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CEREAL CHEMISTRY

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THE RELATIVE EFFECTS OF ENZYMATIC AND PHYSICAL CHANGES DURING STORAGE ON THE CULINARY PROPERTIES OF RICE[1]

H. S. R. DESIKACHAR AND V. SUBRAHMANYAN

ABSTRACT

The amylases were destroyed during the first 5 minutes of cooking milled rice grains, but the solids extracted by the cooking water continued to increase as cooking progressed. The inhibition of amylase action by mercuric salts did not improve the cooking quality of new rice; treatment with formalin or steam-curing of the freshly harvested rice did. The cooking of old rice in amylase solution did not affect its cooking quality.

In aqueous suspensions, fresh-rice flour took more time to settle and left the supernatant liquid more turbid than old-rice flour under similar conditions.

The physical changes that occur during storage have to be considered in explaining the improved cooking quality of old rice.

The improvement in the culinary properties and certain changes in the physicochemical properties of the starch occurring during storage of rice have been reported earlier (4). Two theories have been proposed to explain the better cooking property of the stored rice. The lowering in amylase activity during storage is considered by Sreenivasan (10,11) to be the factor responsible for the improved cooking quality of old rice. A change in the colloidal condition of the rice from the sol to the gel state during storage is believed by Rao (7) to bring about the better cooking quality in the stored grain. The object of the present study was to ascertain the relative importance of these biological and physical changes in determining and explaining the better cooking characteristics of the old grain. The effect of amylases during cooking was studied by determining their rate of inactivation during cooking of new rice. The effect of chemical inactivation of amylases on the culinary quality of the rice and of cooking rice in amylase solutions was also determined. To get some idea as to

[1] Manuscript received June 5, 1958. Communication from the Division of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, India.

the extent to which physical factors could modify the cooking quality of the rice, some changes in the colloidal property of the rice as well as the solubility of starch in perchloric acid were investigated. The effect of soaking rice in certain solvents on its cooking characteristics was also studied.

The differences in cooking quality between old and new rice relate to the lower swelling capacity of the latter and its tendency to yield a thick viscous gruel during cooking. The term *cooking quality* in the present study has therefore been used in a rather restricted sense to denote these two culinary characteristics only.

Materials

Three varieties of rice, Co-11, GEB-24, and S-749, grown locally and representing typical short, medium, and long-grained varieties, respectively, were used in the present study. Rice samples milled from freshly harvested paddy were the source of new rice; that obtained from paddy of the same variety stored for one year served as the source of old rice. Essentially similar differences existed between the new and old samples in the three varieties of rice. The effect of different treatments was also similar in the three varieties tested; hence, results will be reported here for only one variety of milled rice: Co-11 – Bangar Sanna – a fine-grained variety obtained from a paddy breeding station. The old rice sample had the composition: moisture, 12.2%; protein, 5.2%; ether extractives, 2.1%; calcium 9.6 mg.%; and phosphorus, 226 mg.%. The new rice, after drying to the same moisture content as above, had also the same chemical composition.

Methods

Swelling Quality of Rice. Ten-gram rice samples were cooked for 30 minutes in a steam cooker in 50 ml. of water in graduated boiling tubes, the excess gruel was strained off, and the volume and weight of the cooked rice were determined. The effect of cooking the rice in a limited amount of water (two parts) on its swelling quality was also determined in certain cases.

Enzyme Inactivation during Cooking. New rice which is richer in amylases than old stored rice was used for these studies. Ten-gram samples of rice were cooked for different periods in 50 ml. of water on an electric hot plate and then immediately homogenized along with the gruel in a Waring Blendor with 50 ml. of M/15 phosphate buffer of pH 7.0. The homogenate was centrifuged, and the centrifugate was used as a source of alpha- and beta-amylases. For determination of alpha-amylase, 10 ml. of 2% soluble starch solution buffered

with an equal volume of phosphate buffer of pH 7.0 were incubated at 37°C. with 2.5 ml. of the enzyme extract, and, at the end of 18 hours, 1 ml. of the mixture was diluted to 150 ml.; 1 ml. of 10*N* sulfuric acid and 1 ml. of N/10 iodine solution were added and the solution made up to 250 ml. The percent fall in blue color of the starch substrate as measured by a Klett-Summerson photoelectric colorimeter (using red filter no. 66) was taken as a measure of alpha-amylase activity. The beta-amylase activity was determined by measuring the release of reducing sugars when 10 ml. of starch solution and 10 ml. of M/5 acetate buffer (pH 4.6) were incubated at 37°C. for 18 hours with 5 ml. of enzyme extract (Giri and Sreenivasan, 6). Reducing sugars were determined by Somogyi's method (9). At each stage of cooking, as employed in the above studies, solids lost by the rice in the gruel were also determined in separate samples of rice.

Chemical Inactivation by Mercuric Salts. Ten grams of fresh rice were cooked in 50 ml. of 0.2% mercuric chloride and mercuric acetate solutions, to inactivate the amylases (1), and the swelling number of the rice was determined according to Rao (8).

Cooking of Rice in Amylase Solutions. Centrifuged human saliva diluted with 20 volumes of water and also an extract obtained by shaking new-rice flour with five parts of water overnight were used as a source of the amylases. Ten grams of rice were soaked in 50 ml. of the above enzyme extracts for 30 minutes and then cooked in the same solution by steaming for 30 minutes. The swelling number and the loss of gruel solids were determined after cooking.

Treatment with Certain Solvents. New rice was soaked overnight (16 hours) at laboratory temperature (25°–26°C.) in chloroform, 95% ethyl alcohol, and 10% formalin, strained and freed of adhering residual solvent by thorough washing with water. Rice thus treated was then cooked in five parts of water as usual and the swelling quality determined.

Solubility of Rice Starch in Perchloric Acid. Two grams of rice flour passed through 100-mesh sieve were extracted overnight with 50 ml. perchloric acid solution of different concentrations at laboratory temperature, and the starch in the filtrate was determined by precipitation with alcohol (90% V/V), weighing the precipitate after repeated washing with alcohol, and drying to constant weight at 105°C.

Settling Characteristics of Raw and Boiled Rice Flour Suspensions. One percent suspensions of fresh- and old-rice flour obtained by powdering and then passing through a 100-mesh sieve were allowed to settle at 25°–26°C. in 100-ml. graduated cylinders of the same diameter,

and the time needed for settling of the suspended particles was noted. The time at which clear demarcation into two separate layers (of settled flour and top supernatant) became visible was noted. The mixture was allowed to settle further without disturbance for 2 hours, and the turbidity in the top layers was measured in a Klett-Summerson photoelectric colorimeter using blue filter no. 42. Similar observations were made on the suspensions, which had been boiled for 10 minutes and then cooled to laboratory temperature (25°–26°C.).

Results and Discussion

The fact that old stored rice absorbs more water during cooking and hence increases in both weight and volume has been observed by earlier workers. Further interesting observations made in the present study (Table I) show that the improved swelling characteristics of old

TABLE I

BULK DENSITY OF COOKED RICE
(Ten grams rice cooked in steam for 30 minutes)

	Cooked in Excess Water and Gruel Discarded			Cooked in Two Volumes Water		
	Weight	Vol.	Bulk Density	Weight	Vol.	Bulk Density
	g	*ml*		*g*	*ml*	
New	35.3	42	0.84	30.5	29	1.05
Old	42.9	55	0.78	30.5	37	0.83

rice can be considered to be due to two distinct factors: It has the capacity to imbibe more water than the new rice, contributing to an increase in both weight and volume. It also has the ability to swell more in volume than new rice at constant weight, and this factor is responsible for the fluffiness and lower bulk density of cooked old rice as compared with new rice.

Data presented in Table II show that within the first 5 minutes of cooking, practically all the alpha- and beta-amylases present in the rice grain were inactivated. The loss of solids into the cooking water, which is an indication of its tendency to become pasty during cooking, has also been determined at different stages of cooking. It is clear from the data (Table II) that even after the enzymes were inactivated, the grain continued to lose solids into the cooking water. The rate of loss of solids in the gruel is, in fact, greater in the later stages of cooking than in the first few minutes of boiling when the amylases may be expected to exert their action. When mercuric salts were added to the cooking water, the cooking quality of new rice was not improved. (The swelling number of control untreated sample was 2.6;

TABLE II

DESTRUCTION OF α- AND β-AMYLASES IN RELATION TO PASTINESS DURING COOKING OF FRESH RICE

TIME OF COOKING	AMYLASE ACTIVITY		PASTINESS AS MEASURED BY	
	Alpha (as Percent Fall in Blue Value)	Beta (as mg. Maltose Released)	Solids Leached out in Gruel	Solids Leached out between Successive Heating Periods
minutes			%	%
0	83.7	7.9	0.17	...
3	25.0	4.5	0.42	0.25
5	Nil	0.7	0.51	0.09
10	Nil [a]	Nil	0.74	0.23
15	Nil [a]	Nil	1.17	0.37
20	Nil [a]	Nil	1.51	0.34

[a] A slight increase over original blue color was observed in these cases. This might be due to some physical changes in the gelatinized starch present in the enzyme extract added to the reaction mixture.

that of samples cooked in mercuric chloride and acetate solutions was 2.5 and 2.6, respectively.) When new and old rice were cooked in amylase solutions, it was found that the cooking quality of the rice samples was not materially affected by the enzymes (Table III). This

TABLE III

EFFECT OF ADDITION OF AMYLASES ON THE COOKING QUALITY OF RICE

TREATMENT	OLD RICE		NEW RICE	
	Swelling[a] No.	Relative Loss of Gruel Solids	Swelling[a] No.	Relative Loss of Gruel Solids
		%		%
Control	3.3	0.26	2.5	0.35
Control + salivary amylase	3.3	0.26	2.7	0.37
Control + mixed amylases from new rice flour	3.3	0.28	2.7	0.38

[a] Ratio of weight of water absorbed during cooking to weight of raw rice.

was so although the rice samples were soaked previously for 30 minutes in the enzyme extract and slowly cooked in the same solution to give maximum scope for enzymes to react. These facts, taken together, indicate that factors apart from the amylase systems present in rice are important in determining the cooking quality of the rice.

Among solvents which were tried to alter the native physical condition of the starch, chloroform and alcohol did not improve the cooking quality to a noticeable extent. Prior soaking of the new rice in 10% formalin overnight (16 hours), however, enhanced the swelling quality of new rice to the same level as that of old rice, the swelling numbers of new, old, and formalin-treated new rice being 2.5, 3.3, and

3.3, respectively. Also, the cooked grains did not stick to one another. Formalin is known to bring about cross-linking across starch molecules and also to combine with proteins and is used for this reason as a structural fixative agent. The fact that formalin improved the cooking quality of the new rice therefore indicates that the better cooking quality of the old rice could be due to its hardening during storage. In further support of this, 100 g. of new and old rice grains were extracted with water by gentle shaking in a mechanical shaker for 2 hours, and more of solids were found to be extracted from the new rice grains (2.16 g. and 1.76 g. from new and old rice, respectively). Testing the hardness of new and old rice (in the same variety and dried to the same moisture content) in a stiffness or flat-crush testing apparatus (supplied by Gaydon & Co., England, and used in testing of packaging materials) gave indications that the old stored grain was harder than the new grain.

Extraction of new- and old-rice flour with 3% sodium chloride solution showed that the protein in the old rice was less soluble and had suffered denaturation to some extent. Possibility of the existence of similar differences in the solubility of starch in perchloric acid of different concentrations was therefore studied. Data presented in Table IV have not, however, shown any clear-cut trends, although

TABLE IV

SOLUBILITY OF STARCH IN NEW AND OLD RICE IN PERCHLORIC ACID

PERCHLORIC ACID USED FOR EXTRACTION (WT/VOL.)	WEIGHT OF STARCH EXTRACTED	
	New	Old
%	*mg*	*mg*
9.2	6.2	5.7
11.5	45.0	32.5
13.8	620.0	622.5
16.1	687.3	671.2

there are indications that the starch in the old grain is less soluble than that in the new grain. Further experiments under more controlled conditions and with selective solvents for the amylose and amylopectin constituents separately are necessary to confirm the small differences in solubility observed in the present experiments.

Studies on the settling characteristics of rice flour showed that the particles in the new-rice flour tended to settle more slowly than those in the old-rice flour (Table V). The supernatant in the case of new-rice flour was also more turbid. The same was true for the cooked suspensions, too. The flocculent nature of the particles in the new rice was also indicated by the fact that, even after standing for 24 hours, the

TABLE V

TURBIDITY AND SETTLING CHARACTERISTICS OF SUSPENSIONS OF NEW AND OLD RICE FLOUR

	TURBIDITY READING OF SUPERNATANT AFTER SETTLING FOR 2 HOURS		TIME NEEDED FOR SEPARATION INTO TWO LAYERS WHEN ALLOWED TO SETTLE UNDER GRAVITY	
	Uncooked	Cooked	Uncooked	Cooked
			minutes	*minutes*
New	31	192	10	7
Old	13	157	2	1½

volume occupied by the particles that had settled to the bottom was more in the case of new rice. The faster settling rate of particles in the case of old rice could be due either to a change in the property of the rice or to the lower viscosity of the supernatant fluid (4).

Conclusion

Alpha-amylase in cereals, as in rice (unpublished work), is generally known to be relatively stable to high temperatures. The possibility, therefore, existed that during the cooking of intact rice grains, before the inside of the grain could attain the inactivation temperature, the enzyme may act on the starch to influence adversely the cooking quality of the rice. The present study has, however, shown that both the alpha- and beta-amylases are destroyed within the first 5 minutes of cooking of rice grains. This fact, together with the failure to improve the cooking quality of new rice by chemical inactivation of the amylases or to induce pastiness in old rice by the addition of amylase to the cooking water, shows that the amylases do not play a significant role in determining the cooking quality of the rice. The relative differences in culinary quality between new and old rice cannot therefore be explained on the basis of the difference in their amylase activities.

Changes in the solubility and settling characteristics of rice observed in the present study lend further support to the physical theory of sol-gel transformation earlier proposed by Rao (7). A mild form of "wet-heat treatment" has been proposed and successfully applied as a curing method to improve the cooking quality of new rice (5). Parboiling of paddy, which uses a similar form of heat-treatment, is also known to modify the colloidal property of the rice to a gel state (8). It is, therefore, likely that the physical changes during storage bring about a hardening in structure and thus improve the quality of the grain. The fact that formaldehyde, which hardens the structure of tissues by formation of cross links, can also bring about improve-

ment in the cooking quality of the new grain gives further evidence to this possibility.

Reference can be made here to the changes responsible for the staling of bread (2). Whereas the changes that occur during holding of the bread are undesirable, storage changes in the case of the rice grain are desired by consumers. The changes in the physical properties of rice observed here are analogous, at least partially, to the changes observed during the staling of bread. The lower volume of sediment when aqueous suspensions of stale bread crumbs or stored rice particles are allowed to settle as compared with fresh bread or rice particles, respectively, is an instance of comparison (3). Another common feature is the tendency to form lumps during hydration (munching of bread or cooking of rice). This may be related to the difficulty in the digestion of fresh bread or rice. A decrease in the amount of soluble amylose is also observed during the process of bread staling and storage of rice (3,4). A difference between the two processes, however, relates to the capacity for water uptake. Stale bread crumbs have a lower capacity to absorb water and swell (3); stored rice absorbs more water during cooking than fresh rice although, when soaked in cold water or exposed to a humid atmposphere, fresh rice imbibes a slightly larger amount of water than old rice (4). It is proposed to investigate further similarities or differences that may exist between the changes during the two processes.

Literature Cited

1. Bailey, J. M., Thomas, Gwen J., and Whelan, W. J. Selective inhibition of enzymic impurities associated with potato phosphorylase. Biochem. J. **49:** lvi-lvii (1951).
2. Bice, C. W., and Geddes, W. F. The role of starch in bread staling. *In* Starch and its derivatives, ed. by J. A. Radley; vol. II. Chapman & Hall: London (1953).
3. Cathcart, W. H. Review of progress in research on bread staling. Cereal Chem. **17:** 100–121 (1940).
4. Desikachar, H. S. R. Changes leading to improved culinary properties of rice on storage. Cereal Chem. **33:** 324–328 (1956).
5. Desikachar, H. S. R., and Subrahmanyan, V. The curing of freshly harvested paddy. Parts I and II. J. Sci. Industrial Research (India) **16A:** 365–370 (1957).
6. Giri, K. V., and Sreenivasan, A. Das Amylasesystem des Reiskornes während des Reifens und Keimens. Biochem. Z. **289:** 155–166 (1937).
7. Sanjiva Rao, B. Investigations on rice. Current Sci. **6:** 446–447 (1938).
8. Sanjiva Rao, B., Vasudeva Murthy, A. R., and Subrahmanyan, R. S. The amylose and the amylopectin contents of rice and their influence on the cooking quality of the cereal. Proc. Indian Acad. Sci. **36:** 70–80 (1952).
9. Somogyi, M. Notes on sugar determination. J. Biol. Chem. **195:** 19–23 (1952).
10. Sreenivasan, A. Studies on quality in rice. IV. Storage changes in rice after harvest. Indian J. Agr. Sci. **9:** 208–222 (1939).
11. Sreenivasan, A. Veränderungen der enzymatischen Hydrolyse von Stärke im Reis während der Lagerung. Biochem. Z. **301:** 210–218 (1939).

VITAMIN CONTENTS OF AIR-CLASSIFIED HIGH- AND LOW-PROTEIN FLOUR FRACTIONS[1]

C. R. JONES,[2] J. R. FRASER,[3] and T. MORAN[2]

ABSTRACT

The thiamine content of the fine (0-17μ) fraction, obtained by air-classifying a flour laboratory-milled from soft English wheat, was similar to that of the initial flour, while those of the medium (17-35μ) and the coarse (over 35μ) fractions were respectively rather lower and rather higher. With corresponding fractions from a hard English wheat, on the other hand, the value for the fine fraction was much higher and that for the coarse rather lower. The effect of grinding the flour with pinned disks, prior to air-classification, was to reduce the thiamine content of the coarse fraction and, in the case of the soft flour, to raise markedly that of the fine fraction. With the hard flour, the high thiamine content of the fine fraction was maintained, while (as with the soft flour) the yield of this fraction was greatly increased by the grinding.

The levels of niacin in the soft flour were not markedly changed by air-classification, with or without grinding, but with the hard flour, relatively high levels were found in the fine fractions. The riboflavin contents of the fine fractions from both flours were relatively high. The pyridoxine levels in the fine fractions were markedly high with the hard, but only slightly high with the soft, flour. With pantothenic acid, while the corresponding rise in levels was marked with the hard flour, it was not shown at all with the soft.

These effects may be largely explained on the basis of the thiamine and niacin contents of scutellum, aleurone layer, and endosperm present in the flours. These values, known from previous dissection studies, indicate that the initial flours both contained about 0.05% of scutellum, while the contents of aleurone layer were 0.28% in the hard and only 0.07% in the soft. Grinding the flours with pinned disks caused the scutellum and aleurone fragments to be shattered, so that the proportion present as particles under 17 microns rose from about 10% of the total present in the flour to 55 and 100%, respectively, with the hard flour, and to 40 and 60% with the soft. As a result, the scutellum contents of the fine fractions from the ground soft and hard flours were about 0.8 and 1.2%, and the aleurone contents, 0.2 and 1.4%, respectively. Fiber contents indicated that the aleurone was present in the detached form, free from outer parts of the bran, in the fine fractions. The contents of scutellum and aleurone mentioned account largely for the increased ash content in the fine fractions, the increase being particularly high in the case of the hard flour. They also account very largely for differences in contents of pyridoxine, pantothenic acid, and riboflavin between the fractions of different finenesses, except that, with the soft flour only, protein and riboflavin contents in the endosperm appeared to be directly related.

Great interest is now being taken in the possibilities of separating flour, by means of air-classification, into different portions, of high and low protein contents respectively. Jones, Halton, and Stevens (10) have described the mechanism and the principal effects of the separation,

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which depends on the fact that, during milling, some of the interstitial protein of the endosperm breaks up into fragments less than 15 microns in length. These fragments may accordingly be separated from the bulk of the starch granules, which exceed 15 microns in size. The proportion of separable protein may be increased by means of suitable pregrinding of the flour; this has the effect of disintegrating the coarser particles of endosperm which are present to varying extents in all flours. The effect of the grinding, and hence the scope of the whole process, is greater with softer flours such as those from some types of English wheats (which are often of relatively low protein content). The effect of the protein displacement is of potential practical interest in relation to bread- and cake-making qualities of the products. Any process of protein displacement is clearly also of potential nutritional significance, and on the same score it seemed desirable to ascertain whether corresponding displacement of various B-vitamins occurred.

Materials and Methods

Flour Samples. Two samples of flour were prepared by means of the Buhler laboratory mill from soft and hard English wheats respectively. The soft wheat, of low protein content, was a mixture of types (50% Cappelle, 25% Alba (white), 25% Peko) which would conventionally be regarded as suitable for making biscuits (cookies). To minimize bran contamination, the severity of the milling process was limited; the difference in extraction rates obtained with the two wheats reflected the difference in their natural yielding capacities. The yields and color grade values of the flours were: soft, 65%, 2.6; hard, 69%, 3.2.

Air-classification of the flours, into fractions consisting of particles lying between specified sizes, was effected by using a No. 132 Mikroplex Spiral Air Classifier on the lines described by Jones *et al.* (10).

Grinding of certain flour samples prior to air-classification was performed with a No. 4 Kek pinned-disk grinder (described by Jones *et al.*, 10), the shaft of which was driven at 1720 r.p.m. (giving a disk speed of about 11,000 r.p.m.).

Flour color grade values were determined by means of the Kent-Jones and Martin Color grader (11). *Ash* was determined on a 5-g. sample incinerated overnight at 600°C. in a silica dish. *Nitrogen*, determined by the Kjeldahl-Gunning-Arnold method, was converted to protein by the factor 5.7. Other determinations were made as follows: *thiamine*, by a thiochrome method based on the work of Ridyard (13); *fiber*, *riboflavin*, and *niacin* by the methods recommended for flour by the Analytical Methods Committee of the Society of Public Analysts (14,15); *pantothenic acid* by an adaptation of the method described by

Barton-Wright (2) in which Takadiastase was used in place of the chick liver enzyme and phosphatase; *pyridoxine*, by a modification of the method of Atkin *et al.* (1), in which the medium prescribed by Jones and Morris (9) was used, the agar being omitted. This method is essentially that described by Clegg and Hinton (3), differing only in the inoculation technique.

Results

Results obtained on variously treated subsamples of the soft flour are shown in Table I and on those of the hard flour in Table II. In each case, in test 1 the unground flour was separated by means of air-classification into three fractions, denoted as fine, medium, and coarse, consisting of particles lying between the size limits shown. The yields and protein contents in test 1 of Table I are in accordance with those reported as typical for a soft flour by Jones *et al.* (10), who also showed that with hard flours (ordinarily milled), as in test 1 of Table

TABLE I

BEHAVIOR OF SOFT ENGLISH FLOUR,[a] WITH RESPECT TO PROTEIN AND VITAMIN SHIFTS DURING AIR-CLASSIFICATION, WITH AND WITHOUT PRIOR GRINDING

FLOUR OR FRACTION	PARTICLE SIZE	YIELD	PROTEIN	THIAMINE	NIACIN	RIBOFLAVIN	PANTOTHENIC ACID	PYRIDOXINE
	μ	%	%	γ/g	γ/g	γ/g	γ/g	γ/g
	Test 1: Unground flour							
Initial flour		100.0	7.0	0.9	5.1	0.32	3.1	0.5
Fine	0–17	13.0	13.4	0.9	...	...	...	...
Medium	17–35	33.0	3.4	0.6	4.5	0.17	2.2	0.3
Coarse	over 35	54.0	7.8	1.1	...	...	...	...
	Test 2: Coarse fraction from test 1 ground with pinned disks, then classified							
Fine	0–17	11.0	14.7	1.9				
Medium	17–35	21.0	5.1	0.9				
Coarse	over 35	22.0	6.4	0.9				
	Test 3: Initial flour ground with pinned disks, then classified by process used in tests 1 and 2							
Fine	0–17	25.0	14.5	1.4	5.9	0.46	3.0	0.6
Medium	17–35	62.0	3.9	0.7	4.7	0.19	2.1	0.4
Coarse	over 35	13.0	5.9	0.7	...	...	...	...
	Test 4: Flour ground as in test 3, then classified with an additional stage							
1st Fine	0–7	7.0	22.5	2.0	6.7	0.63	3.0	0.8
2nd Fine	7–17	18.0	11.4	1.2	...	...	...	...
Medium	17–35	62.0	3.9	0.7	...	...	...	...
Coarse	over 35	13.0	5.9	0.7	...	...	...	...

[a] Values are expressed on a 14% moisture basis. Yields are expressed with respect to 100 parts of initial flour.

II, the yield of the fine fraction is lower, and the difference between its protein content and that of the initial flour (expressed as a proportion of that of the initial flour) is less than with soft flours.

In test 2, with both the soft and the hard flour, the coarse fraction from test 1 was ground (as described under "Materials and Methods") and subsequently air-classified. This operation gave a further yield of proteinaceous fine material, a result consistent with those obtained in the tests, numbered 3 in each series, in which the flour as a whole was ground prior to classification. The effect of the grinding on the yields and protein contents of the fractions from the soft flour, as reflected in the differences between the results of tests 1 and 3 of Table I, is closely similar to that reported by Jones *et al.* (10). In experiments with the grinder in these laboratories, it has been noticed that its effect is to increase the combined yields of the two finer fractions, and to decrease correspondingly the yield of the coarser fraction, by roughly the same amount with most types of flour, whether hard or soft. Thus, in the present case, the yields of the coarse fraction in tests 1 and 3 on the harder flour (Table II) showed a fall of 35 parts due to the grind-

TABLE II

BEHAVIOR OF HARD (ATLE) ENGLISH FLOUR WITH RESPECT TO PROTEIN AND VITAMIN SHIFTS DURING AIR-CLASSIFICATION, WITH AND WITHOUT PRIOR GRINDING

FLOUR OR FRACTION	PARTICLE SIZE	YIELD	PROTEIN	THIAMINE	NIACIN	RIBOFLAVIN	PANTOTHENIC ACID	PYRIDOXINE
	μ	%	%	γ/g	γ/g	γ/g	γ/g	γ/g
	Test 1: Unground flour							
Initial flour		100.0	8.8	0.9	6.5	0.27	1.5	0.6
Fine	0–17	3.0	13.0	1.8	13.1	0.49	2.1	1.2
Medium	17–35	15.0	4.3	0.7	5.4	0.19	1.6	0.4
Coarse	over 35	82.0	9.1	0.7	...	...	...	...
	Test 2: Coarse fraction from test 1 ground with pinned disks, then classified							
Fine	0–17	15.0	14.4	1.6				
Medium	17–35	27.0	6.3	0.6				
Coarse	over 35	40.0	9.3	0.4				
	Test 3: Initial flour ground with pinned disks, then classified by process used in tests 1 and 2							
Fine	0–17	20.0	15.0	2.2	13.9	0.51	2.4	1.3
Medium	17–35	33.0	5.8	0.7	4.8	0.20	1.3	0.4
Coarse	over 35	47.0	8.3	0.5	...	...	...	...
	Test 4: Flour ground as in test 3, then classified with an additional stage							
1st Fine	0–7	9.0	17.8	3.1	19.4	0.57	2.7	1.9
2nd Fine	7–17	13.0	12.5	1.4	...	...	...	...
Medium	17–35	33.0	5.6	0.7	...	...	...	...
Coarse	over 35	45.0	8.3	0.5	...	...	...	...

ing, the corresponding fall with the softer flour (Table I) being 41. Since, initially, the soft flour was much less coarse than the hard, the end result is that the ground product from the soft flour contains a higher proportion of fine material than that from the hard.

With both flours, test 4 was carried out similarly to test 3, except that an additional very fine separation (giving a "1st fine fraction") was made. The actual levels of protein in the fine fractions from both soft and hard flours (after grinding) were similar, except in the case of the 1st fines of test 4, where the hard flour did not give so high a level as the soft, no doubt because its protein tended to shatter less finely during grinding. In keeping with our experience with other flours, the spread between the protein contents of the fine and medium fractions, following grinding, was rather less with the hard than with the soft flour.

Thiamine Contents. Although the soft and hard flours are similar initially in thiamine content, they differ markedly in respect to its distribution among the fractions of different particle sizes. With the unground soft flour, the thiamine content of the fine fraction is similar to that of the initial flour, but that of the coarse is rather higher. With the hard flour, on the other hand, the thiamine content of the fine fraction is much higher than that of the initial flour and that of the coarse is rather lower.

The effect of grinding is to reduce the thiamine content of the coarse fraction and — in the case of the soft flour — to raise markedly that of the fine fraction. With the hard flour, the high thiamine content of the fine fraction is maintained, while (as with the soft flour) the yield of this fraction is greatly increased by the grinding. The inference is that some vitamin-rich tissue, present, initially, mainly in the coarse fraction, has been broken down relatively finely by the pinned-disk grinding process.

The indication from the results is that any association of thiamine with endosperm protein must, if it exists, be minor in degree, the major effect on the relative thiamine contents of the fractions being due to the behavior of a vitamin-rich nonendosperm tissue. With the soft flour, the evidence points to scutellum as the tissue in question. Stevens (16) has shown in these laboratories that laboratory-milled straight-run white flour may contain 0.3% scutellum. Table III shows that the content of scutellum (calculated from the analytical data[4] in the preceding tables), in the various fractions from the soft flour, ranged from 0.26 to 1.12, that in the initial flour being 0.44%. In particular,

[4] In conjunction with the basic values shown in the footnote to Table III: Simultaneous equations, with aleurone and scutellum contents denoted as x and y respectively, were solved by using the data for niacin and thiamine in that footnote, in conjunction with the niacin and thiamine values for the various

TABLE III

CONTENTS OF SCUTELLUM AND ALEURONE LAYER, CALCULATED[a] FROM THIAMINE AND NIACIN CONTENTS IN CERTAIN FRACTIONS OF TABLES I AND II, AND THEIR EFFECTS ON RIBOFLAVIN CONTENTS

FLOUR OR FRACTION[b] AND DESCRIPTION	ALEURONE	SCUTELLUM	RIBOFLAVIN		
			From Aleurone	From Scutellum	On Scutellum- and Aleurone-free Basis
	%	%	γ/g	γ/g	γ/g
	Soft flour (Table I)				
Initial flour	0.07	0.44	0.01	0.06	0.25
FF from:					
Ground; 7μ; test 4, 1 fine	0.27	1.12	0.02	0.14	0.47
Ground; 17μ; test 3, fine	0.17	0.75	0.02	0.10	0.34
MF from:					
Unground; test 1, medium	nil	0.26	nil	0.03	0.14
Ground; test 3, medium	0.01	0.40	...	0.05	0.14
	Hard flour (Table II)				
Initial flour	0.28	0.45	0.03	0.05	0.19
FF from:					
Unground; 17μ; test 1, fine	1.25	0.86	0.13	0.11	0.25
Ground; 7μ; test 4, 1st fine	2.15	1.64	0.22	0.21	0.14
Ground; 17μ; test 3, fine	1.35	1.20	0.13	0.16	0.22
MF from:					
Unground; test 1, medium	0.12	0.30	0.02	0.04	0.13
Ground; test 3, medium	nil	0.32	nil	0.04	0.16

[a] The following basic values were taken (ref. 12), in γ/g:

	In aleurone layer	*In endosperm*	*In scutellum*
Niacin	670.0	4.5	38.0
Thiamine	16.5	0.2	156.0
Riboflavin	10.0	0.7	13.0

[b] FF = fine fractions; MF = medium fractions.

the calculated scutellum contents for the fine fractions from test 1 (not shown in Table III) and from test 3 were 0.45 and 0.75%; and here independent confirmation was available, based on the finding by Daniels (4) that MHQ (methoxyhyroquinone) glycosides are highly concentrated in the germ of the wheat grain, the contents in germ and endosperm being respectively about 4,000 and 12γ per g. Determinations on the fine fractions from test 1 and test 3 gave respectively 12 and 24γ per g. If it is assumed that the value 4,000 applies equally to scutellum and to whole germ, the difference between the values just given would correspond to a difference of 0.3% in scutellum content. This is in good agreement with the difference between the

flour samples shown in Tables I and II. In framing the equations, the proportion (%) of endosperm in the samples was assumed to be $100 - x - y$.

For example, the equations for the fine fraction (designated in Table III, in line 4 below the headings as "test 3, fine"), which was separated from the ground soft flour at 17 microns, were:

$$670x + 38y + 4.5\ (100 - x - y) = 590 \quad (1)$$
$$16.5x + 156y + 0.2\ (100 - x - y) = 140 \quad (2)$$

from which x is 0.17, and y is 0.75, as shown in Table III.

values of 0.45 and 0.75 calculated from the thiamine contents.

With the harder flour the small amount of the fine fraction initially present contains a relatively high concentration of scutellum, and this concentration is raised more markedly by grinding than it is with the soft flour. Other tests (not fully reported here) on a still harder type of wheat (Svenno) showed the following scutellum contents:

	%
Initial flour (Svenno)	0.64
Fine fraction from unground flour	2.3
Fine fraction from ground flour	2.3

No doubt owing to the relative hardness, and exceptional resistance to reduction, of Svenno wheat endosperm, scutellum enters the flour during milling to a rather greater extent than with the other wheats. In this case, although the pinned-disk grinding process did not raise the concentration of scutellum in the fine fraction, it increased the extent of shattering of the scutellum in the total flour fourfold (since the yield of the fine fraction was increased fourfold).

Niacin Contents. Tables I and II show a further marked differentiation between soft and hard flours in respect to the levels of niacin in the fine fractions—which are much higher with the hard than with the soft flours. Niacin has been shown (5) to be highly concentrated in the aleurone layer, approximate figures for the contents in aleurone layer and endosperm being, respectively, 670 and 5γ per g. The figures in Tables I and II suggest, therefore, that aleurone layer is present to a much greater extent in flours milled from hard wheats than in those from soft, and that it is largely reduced to a finely divided form by the special grinding process. Table III shows the contents of aleurone layer in the present samples, calculated (as described under "Thiamine Contents") from the available data for niacin and thiamine. These reach the rather surprisingly high levels of 1 to 2%, or even more, in fine fractions from the hard wheat, whereas with the soft wheat they are only of the order of 0.2 to 0.3%.

Consideration of the figures further suggests that, with the hard flour, the application of the special grinding process causes all the aleurone layer present to be shattered to such an extent that it enters entirely into the fine fraction (under 17 microns). Thus:

	Sample	*Initial Flour (Atle)*	*Fine Fractions*	
			From Unground Flour	*From Ground Flour*
		%	%	%
a)	Content of aleurone	0.28	1.25	1.35
b)	Yield of fraction	100.0	3.0	20.0
	a × b/100	0.28	0.038	0.27

Calculations on similar lines show that with the soft flour only about half the relatively small amount of aleurone layer present is caused by the special grinding process to enter the fine fraction.

Furthermore, the high content of aleurone layer under discussion must refer to detached aleurone layer – free from the outer layers of the grain (to which it remains attached when present in the by-product of milling known as bran). This is evident from consideration of the figures in Table IV for fiber and ash, determined on certain of the samples of Tables I and II. From the work of Hinton (7), the ash contents of aleurone layer and scutellum may be taken, respectively, as approximately 16 and 7%. On this basis the ash contents, shown in Table IV, support the conclusions already drawn (from the vitamin figures) as to the relative contents of scutellum and aleurone layer in the various fractions.

TABLE IV

ASH AND FIBER FIGURES FOR CERTAIN OF THE SAMPLES LISTED IN THE PRECEDING TABLES, IN RELATION TO CONTENTS OF ALEURONE LAYER AND SCUTELLUM

	Type of Flour Initially			
	Soft		Hard (Atle)	
	Initial Flour (Table I)	Ground, Fine Fraction (Table I, Test 3)	Initial Flour (Table II)	Ground, 1st Fine Fraction (Table II, Test 4)
Contents of:				
Niacin, γ/g	5.1	5.9	6.5	19.4
Ash, %	0.40	0.45	0.40	0.95
Fiber, %	0.07	0.05	0.08	0.04
Aleurone layer, %	0.07	0.17	0.28	2.15
Scutellum, %	0.44	0.75	0.45	1.64
Ash contribution from:				
Aleurone layer, %	0.011	0.027	0.045	0.342
Scutellum, %	0.031	0.053	0.031	0.115
Both above, %	0.042	0.080	0.076	0.457
"Net" ash content, %	0.358	0.370	0.324	0.493

It is evident from Table IV that the relatively very high ash content of the 1st fine fraction from the hard (Atle) wheat is mainly attributable to its high contents of aleurone layer and scutellum. More than one-third of its ash content is contributed by aleurone layer. The low value for its fiber content implies that the aleurone layer is unaccompanied by the other layers of the bran (which have a high fiber content). The "net" ash contents of 0.32 – 0.37% (for the initial flours and the fine fraction from the soft wheat) are such as might reasonably be expected for flour produced by the reduction of substantially pure endosperm.

Other Vitamins. Riboflavin: It is evident from Tables I and II that the contents of riboflavin respond very differently from those of both thiamine and niacin to fractionation by particle size, to the special grinding treatment, and to change in type of wheat. At first glance it might be thought that the trends shown by the fractions from the soft and from the hard flour are much alike. However, in some fractions a considerable part of the riboflavin content is due to the proportions of scutellum and of aleurone layer they contain, and if this is allowed for, as in Table III, the trends of the "net" riboflavin contents (i.e., the contents in the endosperm alone) are seen to differ markedly as between the two wheat types. The fractions from the soft wheat show a fairly regular increase in riboflavin content with increase in protein content, but with the hard wheat there is no real indication of this. The reason for this difference is not clear.

Pantothenic Acid and Pyridoxine: Calculations on the same lines as those set out for riboflavin in Table III (using the values for the vitamin contents of different parts of the grain collated by Moran, 12) show that, with the hard flour, the major part of the increased contents of pantothenic acid and pyridoxine in the fine fractions is attributable, as in the case of riboflavin, to their increased contents of scutellum and aleurone. However, some residual trend with increasing protein content of endosperm appears; thus:

Reference to Sample in Table II	*Protein*	*"Net" Pantothenic Acid*	*"Net" Pyridoxine*
	%	γ/g	γ/g
Test 3, medium	5.8	1.26	0.33
Initial flour	8.8	1.32	0.40
Test 1, fine	13.0	1.46	0.56
Test 3, fine	15.0	1.66	0.55
Test 4, 1st fine	17.8	1.56	0.78

With the soft flour, however, this trend does not appear; virtually the whole of the increase in pyridoxine content shown by the fine fractions is attributable to increased contents of scutellum and aleurone; on the other hand, the fine fractions do not show an increased content of pantothenic acid.[5] A possible explanation of this difference in behavior between the soft and hard wheats would be that the values used in the calculation, for the pantothenic acid and pyridoxine contents of aleurone, and possibly of scutellum, apply well to the soft wheat but require some adjustment in the case of the Atle wheat. The residual trend noted above in the endosperm of the Atle samples would

[5] It is, however, curious that the content of pantothenic acid in the initial flour should be twice as high as in the hard flour.

disappear, on the assumption that the pantothenic acid content of the aleurone layer in Atle wheat were 59 instead of the value of 45γ per g. (12) assumed for the soft English. The same consideration would apply to the pyridoxine values if the content in the aleurone of Atle wheat were 54 instead of 36, or alternatively if, say, those in the aleurone and scutellum were 45 and 35 instead of 36 and 23 respectively. The case of the riboflavin can hardly be explained in this way, however, because the adjustment required to the value assumed for the content in the scutellum of the soft English wheat would appear to be much too great.

Literature Cited

1. Atkin, L., Schultz, A. S., Williams, W. L., and Frey, C. N. Yeast microbiological methods for determination of vitamins. Pyridoxine. Ind. Eng. Chem., Anal. Ed. **15:** 141–144 (1943).
2. Barton-Wright, E. C. The theory and practice of the microbiological assay of the vitamin-B complex; together with the assay of selected amino acids and potassium. Analyst **70:** 283–295 (1945).
3. Clegg, K. M., and Hinton, J. J. C. The microbiological determination of vitamin B_6 in wheaten flour, and in fractions of the wheat grain. J. Sci. Food Agr. **9:** 717–721 (1958).
4. Daniels, D. G. H. The estimation and location of methoxyhydroquinone glycosides in the wheat grain. Cereal Chem. **36:** 32–41 (1959).
5. Heathcote, J. G., Hinton, J. J. C., and Shaw, B. The distribution of nicotinic acid in wheat and maize. Proc. Roy. Soc. (London) **139B:** 276–287 (1952).
6. Hinton, J. J. C. The distribution of vitamin B_1 and nitrogen in the wheat grain. Proc. Roy. Soc. (London) **134B:** 418–429 (1947).
7. Hinton, J. J. C. The distribution of ash in the wheat kernel. Cereal Chem. **36:** 19–31 (1959).
8. Hinton, J. J. C., Peers, F. G., and Shaw, B. The B-vitamins in wheat: the unique aleurone layer. Nature **172:** 993–995 (1953).
9. Jones, A., and Morris, S. A 24-hour plate assay technique for the vitamin-B complex of yeast. Analyst **75:** 613–621 (1950).
10. Jones, C. R., Halton, P., and Stevens, D. J. The separation of flour into fractions of different protein contents by means of air-classification. J. Biochem. Microbiol. Technol. & Eng. **1:** 77–98 (1959).
11. Kent-Jones, D. W., and Amos, A. J. Modern cereal chemistry (5th. ed.), pp. 613–620. Northern Publ. Co.: Liverpool (1957).
12. Moran, T. Nutritional significance of recent work on wheat, flour and bread. (Table I.) Nutrition Abstr. & Revs. **29:** 1–16 (1959).
13. Ridyard, H. N. The determination of aneurine (Vitamin B_1) in uncooked wheat products. Analyst **74:** 18–24 (1949).
14. Society of Public Analysts, Analytical Methods Committee. Determination of the crude fibre in National flour. Analyst **68:** 276–278 (1943).
15. Society of Public Analysts, Analytical Methods Committee. Report on the micro-biological assay of riboflavine and nicotinic acid. Analyst **71:** 397–406 (1946).
16. Stevens, D. J. The contribution of the germ to the oil content of white flour. Cereal Chem. **36:** 452–461 (1959).

SOY FLOUR AS A WHITE BREAD INGREDIENT

I. Preparation of Raw and Heat-Treated Soy Flours, and Their Effects on Dough and Bread[1]

J. M. POLLOCK[2] AND W. F. GEDDES[3]

ABSTRACT

An experimental soy flour, obtained by hammer-milling decorticated soybeans and defatting with cool petroleum ether, proved similar in nitrogen dispersibility and other analytical characteristics to a commercial defatted flour prepared under mild conditions. Controlled heat-treatments (1 hour, 7.9% moisture) at 75°C. or below had no appreciable effect on nitrogen dispersibility; treatment at 100°C. or above substantially reduced nitrogen dispersibility and materially darkened soy flour color.

Inclusion of raw soy flour in farinograph doughs at levels of 1–5% imparted to normal and rest-period curves the characteristics of a stronger flour, the effect increasing with the soy flour level. Soy flour heated 1 hour at 100°C. showed this property to a lesser degree. In baking tests employing 1 mg. potassium bromate per 100 g. of flour, 1% raw soy flour somewhat improved the bread, but higher levels decreased loaf volume; heated soy flours were still more injurious, in proportion to their degree of heat-treatment. Heat-treatment raised the water absorption of the soy flours in doughs.

Although the nutritional advantages of soy flour have been appreciated by many, its acceptance as a bread ingredient has been rather limited mainly because of functional disadvantages and nonuniformity of soy flours in early stages of their development. The functional problems generally associated in the past with the use of soy flours in bread dough include (a) alteration of absorption, mixing, and machining properties, (b) adverse effects on color and flavor, (c) changes in fermentation rates and (d) effects on the gluten complex, including oxidation requirements (4,5,6). Although improvement in processing methods has steadily reduced these disadvantages, lack of uniformity among commercial soy flours has rendered adaptation to this new ingredient difficult (15,16).

One object of the present work was to assess the baking quality of a specially prepared, unheated soy flour and to determine its effects on dough characteristics. Since soy flours receive widely varying degrees of heat-treatment during processing and in view of the well-known effects of heat on the baking quality of milk (8,9) and on

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wheat flour itself (7), the effect of heat on the baking quality of extracted raw soy flour was also examined.

Materials and Methods

Soy Flour Samples. A sample of unheated, decorticated soy beans, ground in a hammer mill to pass 95.9% through a No. 100 U.S. standard screen,[4] was defatted in 3-kg. batches by exhaustive cold extraction with petroleum ether in a Lloyd percolator. The extraction rate was controlled to hold the temperature between 22° and 29°C. This specially extracted flour was air-dried and pulverized during drying. It was then bolted through a 9 XX grits gauze on a mechanical shaker and blended in a MacLellan mixer.

Commercial soy flours representative of the chief types available on the market were obtained for comparison with the experimental soy flour.[5]

Heat-Treatment of Extracted Raw Soy Flour. To minimize moisture losses during heat-treatments, a modified form of the equipment described by Geddes (7) was used. This consisted of a closed stainless-steel cylinder (11.7 by 39 cm. inside dimensions), arranged to rotate in an oil bath to provide a combined tumbling and end-to-end motion.

The laboratory-defatted raw soy flour (7.9% moisture) was heated in 250-g. quantities at 50°, 75°, 100°, and 125°C. for 1 hour.

Analysis of Commercial and Experimental Soy Flours. The ground, decorticated soybeans, the defatted flour, and the commercial soy flours were analyzed, chiefly by routine methods. Moisture and volatile matter were determined by the method of the National Soybean Processors' Association (14). Total nitrogen was determined as prescribed in section 2.26 of AOAC *Methods* (3), except that, in the distillation, the ammonia was absorbed in 4% boric acid solution and titrated directly with standard acid. Ash content was obtained by the NSPA method (14). To determine fat content, the samples were first dried and the moisture loss determined after heating at 95°–100°C. under less than 100 mm. pressure for 5 hours (2). Fat was then determined on the dried material by the NSPA method (14).

For the determination of reducing sugars, the AACC method (1) was modified as follows: extraction of sugars was begun by adding the prescribed amounts of alcohol and acid buffer solution, then shaking briefly but sharply to suspend the sample. After the immediate addition of sodium tungstate solution to inactivate enzymes, the extraction

[4] Courtesy of the Northern Utilization Research and Development Division, Peoria, Illinois.
[5] Courtesy of the Soya Food Research Council, Washington, D. C.

was completed by shaking for exactly 30 seconds. The reducing power of an aliquot against potassium ferricyanide was then determined as directed in the method. Results were expressed empirically in terms of maltose, although substantially none of the reducing power of soy flour is due to maltose.

Urease activity was estimated[6] as the pH increase resulting upon incubation of 0.200 g. of soy flour with 3% urea solution in 0.05*M* phosphate buffer of pH 7.0 at 30°C. for 30 minutes.

Water dispersibility of the nitrogen in the soy flour samples was determined as a criterion of the influence of processing conditions on the protein. Although this property is considered important in the soy flour industry, no standard method has been accepted. In the present work the "nitrogen dispersibility" was obtained by mechanically shaking a 5-g. sample for 2.5 hours with 100 ml. of distilled water at 26°C., centrifuging, and determining the nitrogen in an aliquot of the centrifugate (3).

Physical Dough Tests. The effects of 1, 3, and 5% levels of raw and heated (1 hour at 100°C.) soy flour were evaluated by replacing a portion of wheat flour with the required weight of soy flour and making farinograph tests (small mixing bowl) in the usual manner (1).

"Rest-period" farinograph curves on the same dough compositions were prepared by mixing to maximum consistency, stopping the machine, and leaving the bowl undisturbed for 1 hour with the temperature controlled at 30°C. The machine was then started and the dough remixed for 2 minutes. Three such rests were employed for each mixture.

The farinograph was used in a different manner to standardize absorption or to determine consistency in complete doughs. The proper absorption of the wheat flour was first established at 60% by "feel." The mixing of all the ingredients employed in the baking formula, scaled down to a total weight of 80 g., yielded a farinograph consistency of 400 units. This consistency reading could be reproduced by inserting in the bowl an 80-g. portion of dough premixed in the McDuffee bowl for 3 minutes. Subsequently, this method was used to determine the baking absorption of wheat flour-soy flour mixtures. On the basis of these absorptions, the "water requirement" of each soy flour was calculated as the ratio of water to soy flour (by weight) required to yield the standard consistency (400 units) in a given wheat flour-soy flour dough.

[6] Courtesy R. E. Anderson, Control Laboratory, Archer-Daniels-Midland Company, Minneapolis, Minnesota.

Experimental Baking Tests. The test formula employed was an average commercial-type formula (Table I) with ingredients in such amounts as to yield 250-g. doughs. The baking procedure (Table I) was of the straight dough type described in *Cereal Laboratory Methods* (1).

TABLE I

BAKING FORMULA AND PROCEDURE FOR 250-GRAM DOUGHS

	BAKING FORMULA	
	Percent of Wheat Flour, 14% Moisture Basis	Weight
		g/mix
Flour [a]	100.0	300.0
Yeast	3.0	9.0
Sugar	5.0	15.0
Salt	2.0	6.0
Dough improver [b]	0.3	0.9
Shortening	2.0	6.0
Water	60.0	180.0
	PROCEDURE	
Mixing time (minutes) [c]	2.5	
Dough weight (g.)	250	
Fermentation at (°C.)	30	
(°F.)	86	
and r.h. (%)	90	
Punched [d] after (minutes)	95	
Molded after (minutes)	25	
Proofing time (minutes)	55	
Oven time at 232°C. (450°F.) (minutes) [e]	25	

[a] Commercial Southwestern flour, 80% baker's patent; ash 0.40%, protein 11.2% (14% moisture basis).
[b] A commercial bread improver containing potassium bromate (0.3%), yeast foods, and an inert ingredient.
[c] Medium speed on Hobart C-10 mixer, equipped with a McDuffee bowl and mixing head.
[d] Punching was done on a National dough sheeter, with rolls set at 9/32-in. For panning, doughs were sheeted twice, at 9/32- and 5/32-in. roll settings. Molding was done by hand.
[e] Baking was carried out in a specially constructed National rotary hearth oven.

Loaf volumes were determined with a National Loaf Volumeter 1 hour after baking, and the loaves were scored the following day for other characteristics.

Results

Analysis of Commercial and Experimental Soy Flours. Analytical data for the commercial and laboratory-prepared soy flours are recorded in Table II. The laboratory-extracted raw soy flour (VI) was similar in several respects to the commercial solvent-extracted flours (II, III). However, the data for urease activity and nitrogen dispersibility, which are highly dependent on degree of heat-treatment, mechanical injury, and other processing factors, revealed marked differences between the commercial flours. Judging from decreased nitrogen

TABLE II

ANALYSIS OF COMMERCIAL AND LABORATORY-PREPARED SOY FLOURS[a]

No.	Nature of Sample	Protein	Ash	Fat	Reducing Sugars[b]	Urease	Nitrogen Dispersible in Water
		%	%	%		*pH units*	% *of total*
I	Ground raw decorticated soybeans	44.1	5.06	23.1	132	1.9	65.2
II	Commercial unheated defatted soy flour (solvent extracted)	56.7	6.26	0.3	178	2.0	77.8
III	Commercial defatted soy flour (solvent extracted)	58.5	6.42	0.6	124	1.7	57.0
IV	Commercial low-fat soy flour (expeller process)	55.5	5.90	4.7	116	0.4	21.4
V	Commercial full-fat soy flour	43.5	4.70	24.8	103	0.2	21.8
VI	Laboratory-defatted raw soy flour[c]	57.0	6.37	0.3	128	...	77.6

[a] Analytical values are expressed on a dry-matter basis and are the means of at least duplicate determinations.
[b] Expressed as mg. maltose per 10 g. soy flour.
[c] Represents Sample No. I extracted with petroleum ether, as described in text.

dispersibility in particular, commercial sample No. III appeared to have been processed under more drastic conditions than No. II. Commercial soy flour II was quite similar to the experimental flour, which was carefully prepared to avoid any heat effect.

Other commercial soy flours were decidedly lower in nitrogen dispersibility and urease activity. These effects presumably are traceable to the expeller method of defatting the low-fat soy flour (IV) and the method of grinding the full-fat product (V). The somewhat lower

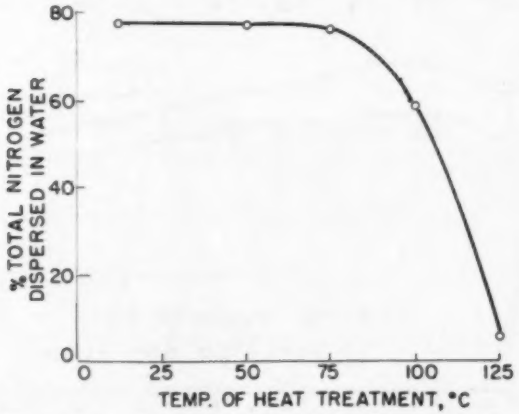

Fig. 1. The effect of controlled heat-treatment on nitrogen dispersibility of experimental, unheated, defatted soy flour. The nitrogen dispersion levels of three commercial soy flours were as follows: unheated, defatted soy flour, 78%; defatted soy flour, 57%; and low-fat soy flour, 21%.

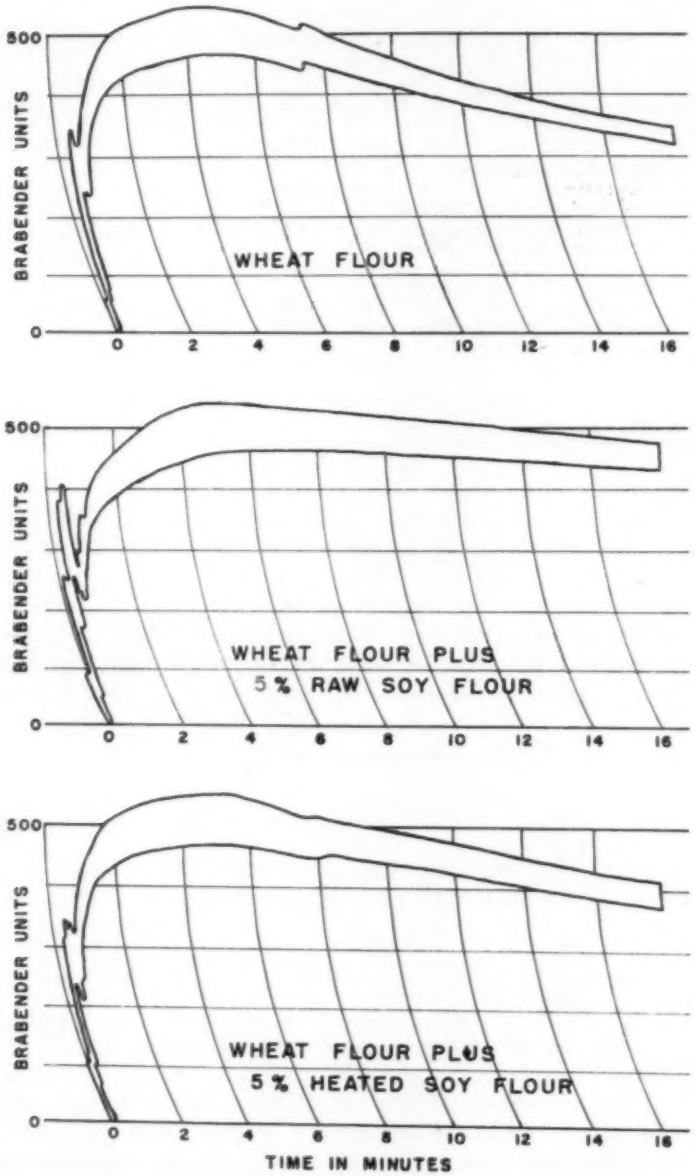

Fig. 2. The effect of raw and heat-treated soy flours on characteristics of the normal farinograph curve.

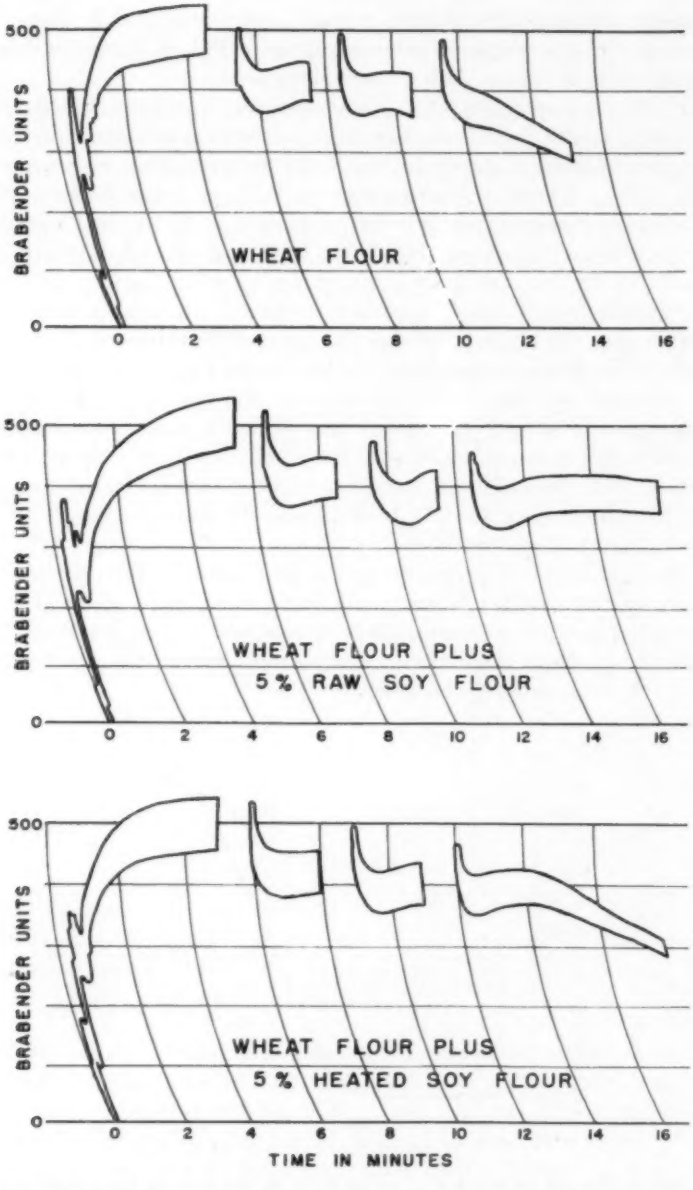

Fig. 3. The effect of raw and heat-treated soy flours on farinograph "rest period" curves.

nitrogen dispersibility of the ground, raw, decorticated beans (I) than of the defatted flour prepared from it (VI) is probably due to interference of the fat with protein dispersion.

Effect of Controlled Heat-Treatment on Nitrogen Dispersibility of Experimental Soy Flour. The influence of heat-treatment on nitrogen dispersibility is shown in Fig. 1. At the prevailing moisture content (7.9%), 1-hour heat-treatments at 75°C. or below had no effect on nitrogen dispersibility; however, treatment at 100°C. had a marked effect, whereas treatment at 125°C. largely destroyed nitrogen dispersibility.

Physical Dough Tests. Normal farinograms for doughs made with and without the addition of raw (5%) and of heat-treated (1 hour at 100°C.) soy flours, respectively, are shown in Fig. 2. As little as 1% of unheated soy flour (VI) imparted to the dough the farinogram characteristics considered typical of a stronger flour. The increased dough stability, curve width, and dough development time were augmented with increased percentages of raw soy flour. Heated soy flour had a smaller effect; the 5% level altered the control curve less than the 1% level of raw soy flour.

Rest-period farinograms, to reveal any delayed effect of these soy flours on the physical properties of the doughs, were made (Fig. 3). The apparent strengthening effect of raw and heated soy flours prevailed throughout three 1-hour rest periods; again, the raw soy flour gave the more pronounced effect.

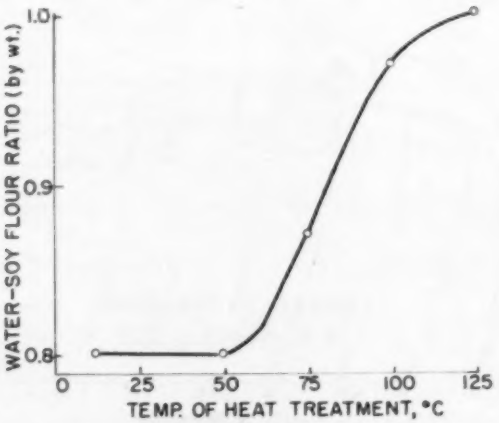

Fig. 4. Effect of heat-treatment of soy flour on the ratio of the weight of additional water to the dry weight of soy flour which is required to yield a dough of standard consistency (400 B.u.) in the farinograph.

The inclusion of soy flour in dough necessitated the use of additional water to obtain a standard farinograph dough consistency. Figure 4 shows the water requirements of raw and heated soy flours, in terms of the ratio of the weight of additional water to the dry weight of soy flour which is required to yield a dough with a consistency of 400 B.u. The water-soy flour ratios probably reflect the influence of heat on water-binding capacity of the protein. Comparison with the nitrogen dispersibility data (Fig. 1) indicates an inverse relationship between nitrogen dispersibility and the water-binding capacity as approximated by the farinograph method.

TABLE III

ABSORPTION AND MEAN LOAF VOLUMES OF LOAVES CONTAINING HEAT-TREATED SOY FLOURS

SAMPLE NO.	TEMP. OF HEAT TREATMENT[a]	SOY FLOUR LEVEL[b]	ABSORPTION	LOAF VOLUME[c]
	°C	%	%	cc
Control		0	60.0	1225
VI	nil	1	60.8	1285**
		3	62.4	1250
		5	64.0	1200
VI-a	50	1	60.8	1220
		3	62.4	1220
		5	64.0	1175**
VI-b	75	1	60.9	1230
		3	62.6	1235
		5	64.3	1170**
VI-c	100	1	61.0	1215
		3	62.9	1185*
		5	64.9	1110**
VI-d	125	1	61.0	1200
		3	63.0	1175**
		5	65.0	1135**

[a] Heat treatments were for 1 hour at 7.9% moisture.
[b] Based on wheat flour at 14% moisture.
[c] Means of duplicates. The standard error (single determination) computed from all sets of duplicate values was 16.1 cc. A minimum difference of 32.2 cc. is required between any two means for them to be considered significantly different; to be regarded as highly significant the differences must equal or exceed 48.3 cc. The loaf volumes which are significantly different from the control at the 5% and 1% points are designated by * and ** respectively.

Baking Tests. The loaf volumes obtained from doughs containing 1, 3, and 5% levels of raw and heated soy flour samples are shown in Table III. Unheated soy flour (VI) at the 1% level gave a significant loaf-volume increase. The higher levels had no appreciable effect; this may be due to the use of a constant level of oxidizing improver in all samples. In general, heated soy flours were deleterious, the effect increasing with severity of heat-treatment. The dough formula

could "carry" 3% of soy flour heated at 50° or 75°C. without a significant decrease in loaf volume; only 1% additions of samples heated at 100° or 125°C. could be tolerated. Although these experimentally heated samples are not comparable with most commercial soy flours, the poor performance in bread of some commercial products, many of which are subjected to considerable heat-treatment, is easily understood.

Crumb color of bread containing any appreciable amount of soy flour has long been a problem. While a 5% level of any soy flour sample in this series was sufficient to cause significant crumb discoloration, samples heated at 100° and 125°C. gave progressively more undesirable colors. Thus, the most marked color development paralleled the most severe loss in nitrogen dispersibility, as shown in Fig. 1.

A slightly different picture was presented by the flavor scores; samples containing the soy flour heated at 100°C. were rated highest. Apparently this treatment was sufficient to dispel the raw "beany" flavor, but not enough to impart the marked toasted flavor characteristic of more severely heated samples.

Discussion

These results are based on dough formulas containing a single level of bromate-containing dough improver, and the relative performance of the soy flours would probably have been altered by employing different amounts of bromate. The work of Finney (6), Bayfield and Swanson (4), and, more recently, of Ofelt *et al.* (15,16) has indicated that with the use of proper amounts of bromate, the effects of good-quality soy flours are without practical significance when used at levels of about 5%.

The beneficial effect of raw soy flours on the stability of the doughs, as measured by the "normal" and "rest-period" farinograms, is quite striking. It is well known that soy beans and many other leguminous seeds contain a trypsin inhibitor (12). Learmonth (10) has shown that aqueous extracts of raw soybeans will inhibit the action of papain on a gelatin substrate and that heating the extract will decrease the inhibition. With the use of the rest-period farinograph technique with fermenting doughs, he showed that aqueous extracts of raw soybeans would decrease the weakening of control doughs (and more particularly of doughs containing added papain) which normally occurs upon extended fermentation (11). Melnick (13) has obtained a patent on the use of an aqueous extract of soy flour which inhibits the degradation of gluten and improves the baking quality of bread doughs containing "immature" flour, wheat germ, or other components that contain proteases or protease activators.

In the present studies, the beneficial effects of 5% raw soy flour on the farinogram were not reflected in improved baking quality. The observation that increasing levels of raw soy flour above 1.0% decreased baking quality suggests that inhibition of proteases was not the principal or sole factor responsible for its effects in doughs.

Acknowledgments

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Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). The Association: St. Paul, Minnesota (1947).
2. Association of Official Agricultural Chemists. Official and tentative methods of analysis (6th ed.). The Association: Washington, D. C. (1945).
3. Association of Official Agricultural Chemists. Official and tentative methods of analysis (7th ed.). The Association: Washington, D. C. (1950).
4. Bayfield, E. G., and Swanson, E. C. Effect of yeast, bromate, and fermentation on bread containing soy flour. Cereal Chem. **23**: 104–113 (1946).
5. Bohn, R. T., and Favor, H. H. Functional properties of soya flour as a bread ingredient. Cereal Chem. **22**: 296–311 (1945).
6. Finney, K. F. Loaf volume potentialities, buffering capacity, and other baking properties of soy flour in blends with spring wheat flour. Cereal Chem. **23**: 96–104 (1946).
7. Geddes, W. F. Chemical and physico-chemical changes induced in wheat and wheat products by elevated temperatures. I. Can. J. Research **1**: 528–557 (1929).
8. Jenness, R. Recent work on the effects of milk in bread. Baker's Digest **28**: 87–91, 103 (Oct. 1954).
9. Larson, B. L., Jenness, R., Geddes, W. F., and Coulter, S. T. An evaluation of the methods used for determining the baking quality of nonfat dry milk solids. Cereal Chem. **28**: 351–370 (1951).
10. Learmonth, E. M. The influence of soya flour on bread doughs. I. A papain-inhibiting factor in soya beans. J. Sci. Food Agr. **2**: 447–449 (1951).
11. Learmonth, E. M. The influence of soya flour on bread doughs. II. The inhibition of the proteolytic enzymes of malt and wheat flours. J. Sci. Food Agr. **3**: 54–59 (1952).
12. Liener, I. E. The effect of heat processing and storage on the nutritive value of proteins of importance in the cereal industry. Trans. Am. Assoc. Cereal Chemists **8**: 162–185 (1950).
13. Melnick, D. Inhibiting protein degradation in food. U. S. Patent 2,670,291 (Feb. 23, 1954) (Chem. Abstracts **48**: 6048b).
14. National Soy Bean Processors' Association. Handbook of analytical methods for soybeans and soybean products (1946).
15. Ofelt, C. W., Smith, A. K., and Derges, R. E. Baking behavior and oxidation requirements of soy flour. I. Commercial full-fat soy flours. Cereal Chem. **31**: 15–22 (1954).
16. Ofelt, C. W., Smith, A. K., and Mills, J. M. Baking behavior and oxidation requirements of soy flour. II. Commercial defatted soy flours. Cereal Chem. **31**: 23–28 (1954).

SOY FLOUR AS A WHITE BREAD INGREDIENT
II. Fractionation of Raw Soy Flour and Effects of the Fractions in Bread[1]

J. M. POLLOCK[2] AND W. F. GEDDES[3]

ABSTRACT

An unheated, defatted soy flour was fractionated by mild solvent separation, precipitation, and dialysis techniques. The baking performance of the flour and its fractions, employed at a level of 3% (wheat flour basis), was tested by a small scale procedure using several levels of potassium bromate. Chemical studies were made on the fractions in an attempt to relate their composition to baking performance. The raw soy flour caused reproducible adverse effects on loaf volume, but appropriate heat treatment improved loaf volume when baked with a satisfactory level of bromate. Heat treatment of several fractions adversely affected loaf volume; others were not significantly influenced, and one was improved.

The most injurious fractions were encountered in the dialysate from the supernatant after precipitation of most of the protein at pH 4.2 from the water-soluble fraction. Still another dialysate fraction contained most of the undesirable flavor, although it was otherwise of fair baking quality. A protein fraction, not precipitated at pH 4.2, was excellent in baking quality and showed high antitryptic activity.

Analyses of various fractions demonstrated that chlorides, sugars, and sulfhydryl groups were not responsible for the poor baking quality of the fractions in which they were found. Effects of fractions on dough pH were eliminated as a cause of poor baking quality. Quantitative analysis of the two most injurious fractions indicated significant levels of zinc, calcium, magnesium, and phosphate ions. In baking tests only zinc and phosphate ions corresponding to the levels in 3% of the most injurious fraction were found harmful. Zinc and phosphate ions in the amounts employed in the baking tests (0.0048 and 0.60% respectively, flour basis) retarded gas production; whereas the adverse effects of raw soy flour itself, and of the acid-precipitable protein, were primarily on gas retention. Thus, the inorganic constituents may be of relatively little importance in the performance of soy flour iself.

In a previous paper the authors reported that the addition of 1% experimentally prepared, defatted, raw soy flour to a Southwestern Bakers' Patent flour somewhat improved loaf volume as determined by a bromate baking formula containing 1 mg. of potassium bromate per 100 g. of flour, whereas higher levels had a deleterious effect when baked with the same bromate dosage (24). The raw soy flour markedly improved the stability of nonfermenting doughs as measured by normal and rest-period farinograms, the extent of the improvement increasing with the soy flour level employed over the range of 1 to 5%.

[1] Manuscript received March 29, 1956. Paper No. 4181, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper is based upon portions of a thesis presented to the Graduate School of the University of Minnesota by J. M. Pollock, in partial fulfillment of the requirements for the Ph.D. degree, April, 1953.

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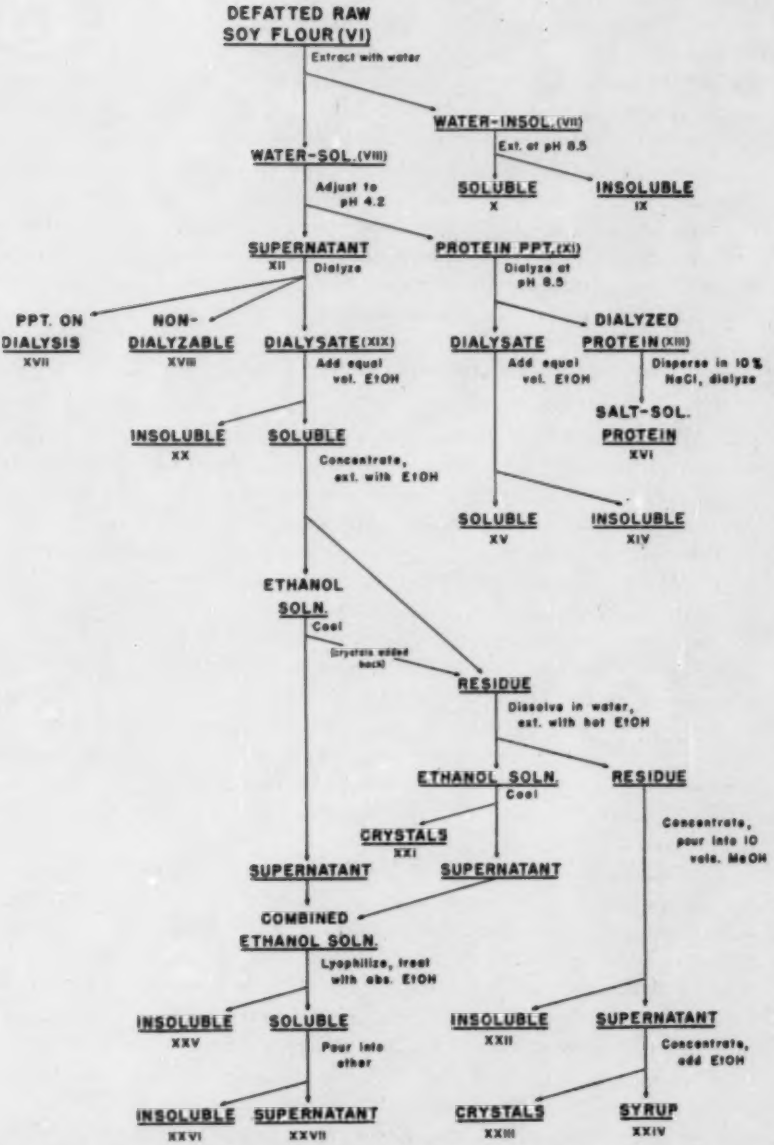

Fig. 1. Schematic outline of fractionation procedure applied to experimental unheated defatted soy flour.

Heat treatment of the soy flour at 100°C. or higher for 1 hour at 7.9% moisture decreased the water dispersibility of the proteins, decreased its baking quality as reflected by the bromate baking test, and decreased its beneficial effect on the stability of doughs to extended mixing in the farinograph.

The experiments reported in the present paper were undertaken to explore the properties of various fractions prepared from soy flour. Through a study of the composition and baking characteristics (as influenced by heat treatment and the presence of an oxidizing improver) of such fractions, it might be possible to eliminate the harmful effects of certain constituents by suitable modifications in processing soy flour or by changes in the bread formula or baking procedure.

Fractionation of Soy Flour

Initial Extraction Steps. The defatted raw soy flour (500 g.) described by Pollock and Geddes (24) was fractionated according to the scheme outlined in Fig. 1. The first step was a 2-hour water extraction at pH 6.5 and 30°C., using a solvent:solid ratio of 20:1 with continuous stirring at medium speed. The thin slurry resulting was centrifuged by three passes through a Sharples supercentrifuge, giving the water-insoluble and water-soluble fractions, designated nos. VII and VIII, respectively. Both fractions were frozen, crushed, and dried from the frozen state in a refrigerated chamber under 1 to 2 mm. pressure, using a small radiant heat input. The water-insoluble fraction (VII) obtained from one batch of soy flour without drying was re-extracted by continuous stirring with 20 parts of very dilute aqueous sodium hydroxide at pH 8.5. After centrifuging at 28,000 r.p.m. (one pass), the centrifugate was adjusted back to pH 6.5 with dilute hydrochloric acid; the insoluble (IX) and soluble (X) fractions were then dried in the frozen condition.

Fractionation of Water-Soluble Material: Precipitation of Protein. To precipitate most of the soluble protein, the water-soluble fraction (VIII) obtained from the supercentrifuge was adjusted to a pH of 4.2 with dilute hydrochloric acid solution. The acid-precipitated protein was recovered in a centrifuge, washed, twice redissolved in dilute sodium hydroxide solution at pH 8.5, and reprecipitated. The moist protein was frozen and dried by lyophilization to yield fraction XI.

The supernatant from No. XI was frozen, crushed, and dried giving fraction XII. This material consisted principally of low molecular weight substances and required care in lyophilization.

Additional Protein Purification Measures. A new preparation of moist protein (No. XI), after washing at pH 4.2 and redissolving at

pH 8.5, was placed in sections of Visking casing and dialyzed for 4 days at 2°–4°C. against slightly alkaline solvent. Fresh external medium was provided daily, and the protein solution was protected against bacterial growth by small quantities of chloroform and toluene. The dialyzed protein was precipitated at pH 4.2, centrifuged, quick-frozen, and lyophilized (fraction XIII).

The combined dialysate from No. XIII was concentrated to a small volume under reduced pressure at 30°–35°C. To the concentrated dialysate, an equal volume of 95% ethanol was added, yielding a precipitate (fraction XIV). The supernatant, fraction XV, was concentrated under reduced pressure to remove the ethanol, diluted with water, frozen, and dried.

A second batch of dialyzed protein (No. XIII) was stirred 4 hours with 20 parts of 10% sodium chloride solution at 30°C. The clear supernatant upon centrifuging was dialyzed at 2°–4°C. against frequent changes of eight volumes of distilled water until a negative test for chloride ion was obtained. The protein precipitated during dialysis was centrifuged, frozen, and lyophilized (fraction XVI).

Further Treatment of Supernatant (XII) from Original Protein Precipitation. A fresh portion of XII was dialyzed at 2°–4°C. for 4 days against 1.5 volumes of distilled water, changed daily. The dialysate collected during the first 3 days was retained. During dialysis a trace of solid material precipitated, which was separated by centrifuging, frozen, and lyophilized (fraction XVII). The centrifugate, constituting the remainder of the nondialyzable fraction, was isolated in the same manner (fraction XVIII).

The dialysate portions above were adjusted as obtained to pH 6.5 and concentrated by perstillation in a 40°C. forced-draft oven to about one-sixth their original volumes. The combined concentrated dialysate was frozen and dried in the frozen state without application of heat. The dried material was extremely hygroscopic; since it was impractical to handle, sufficient water was added to give a heavy syrup (fraction XIX).

Fractionation of Dialysate (XIX) with Ethanol. The concentrated dialysate (XIX) from three batches of defatted soy flour was accumulated, and an equal volume of 95% ethanol was added, precipitating fraction XX. The supernatant alcoholic solution was concentrated under reduced pressure to a heavy syrup, which was extracted five times with 95% ethanol at 27°C. Crystalline products separating from the cold ethanol extracts on standing were returned to the residue. The residue was dissolved in water to give a syrup and extracted five times with hot 95% ethanol (70°C.). Cooling of the combined hot

ethanol extracts gave crystalline fraction XXI.

The cold and hot ethanol extracts, when combined and concentrated to a small volume under reduced pressure, separated into immiscible layers. The mixture was dried in the frozen state and the solid treated with absolute ethanol. The ethanol-insoluble residue, being very hygroscopic, was converted to a syrup (fraction XXV) for ease of manipulation. The absolute ethanol solution was again concentrated to a small volume and poured into five volumes of ethyl ether, precipitating fraction XXVI, which was dried to a gummy solid. The ether solution, upon evaporation, yielded a trace of red-brown syrup (fraction XXVII).

Fractionation of Original Ethanol-Insoluble Residue with Methanol. The residue, after extracting the concentrated dialysate fraction with cold and with hot ethanol, was taken up in water to form a light syrup. When poured into ten volumes of methanol, the material gave a bulky precipitate, which was filtered rapidly, washed, and dried over phosphoric anhydride (fraction XXII).

From the filtrate, methanol was removed under reduced pressure. Upon cooling at 2°–4°C., crystals were obtained. The supernatant was concentrated further and ethanol added to incipient crystallization; upon cooling overnight a second crop of crystals was obtained. The procedure was repeated to give a third and fourth crop. All crystalline products were combined to form fraction XXIII. To remove all alcohol, the supernatant was evaporated under reduced pressure to a semisolid syrup and water added to give a medium heavy syrup, which was retained as fraction XXIV.

Heat Treatment of Fractions. The soy flour fractions in general were heat-treated for 1 hour at 100°C., using the apparatus described in a previous paper (24).

Because of the limited quantities and variable nature of the fractions, it was not feasible to bring them all to the same moisture content for heat treatment. The moisture contents at which the fractions were heated are shown in Table II. For certain fractions available in limited supply, the equipment was modified by using a smaller stainless steel cylinder (3.8 by 15.2 cm., inside dimensions) to prevent excessive moisture loss during heat treatment.

Syrupy fractions were heat-treated by exposure in open tubes in an oil bath at 80°C. for 30 minutes with frequent stirring.

Subfractionation Methods

Certain subfractionation techniques, not part of the original procedure, were adopted as results of baking tests and analytical data on

the fractions became available. They were applied to those fractions which proved most active in the baking tests.

Water-Soluble and Insoluble Components of Fraction XX. Fraction XX, shown to be highly injurious to bread quality, was only partially water-soluble. To determine whether the activity lay in the soluble or insoluble portion, a 3.55 g. sample was shaken at 30°C. with four successive 15-ml. portions of distilled water for 30 minutes each. At each stage, the mixture was centrifuged and the supernatant solution removed. The combined supernatant and a slurry of the insoluble residue were frozen and lyophilized to obtain insoluble (XX_1) and soluble (XX_2) subfractions.

Ion Exchange Separation. Fraction XXII, suspected of containing considerable inorganic material, was separated into neutral and combined acidic and basic subfractions by an ion exchange procedure: a 3% solution containing 6 g. of fraction XXII was passed slowly through a column of Amberlite (cationic) resin IR-120 (acid form). The eluate was passed directly through a column of Duolite (anionic) resin A-4 (alkaline form). The resins were washed with six volumes of distilled water; the combined eluate and washings were concentrated under reduced pressure to a medium heavy syrup, which was retained as neutral subfraction $XXII_a$.

The portions exchanged by the respective resin columns were removed by regenerating the columns in the usual manner with hydrochloric acid and sodium hydroxide solutions. The eluates thus contained excess mineral acid and alkali; these were removed by passage again through the appropriate resins. The final eluates were combined, concentrated under reduced pressure, and evaporated to dryness in a vacuum oven at 50°C. and less than 10 mm. pressure. The dried material, the acidic and basic constituents of fraction XXII, was designated $XXII_b$.

Treatment of Fraction XXII with Ethanol-Water Mixtures. In an attempt to separate the components present in fraction XXII by solubility differences, a 40 g. sample was extracted with two portions each of 80, 70, and 60% aqueous ethanol. Each extraction was carried out by shaking 40 minutes, centrifuging, and removing the supernatant. Each pair of extracts was combined and concentrated under reduced pressure to a syrup. The residue, after the 60% ethanol extraction, was separated into a water-soluble portion (retained as a syrup) and an insoluble solid (frozen and lyophilized). Thus, the subfractions obtained were as follows:

Subfraction $XXII_1$	Extracted from XXII by 80% ethanol
Subfraction $XXII_2$	Extracted from XXII by 70% ethanol
Subfraction $XXII_3$	Extracted from XXII by 60% ethanol
Subfraction $XXII_4$	Water-insoluble portion of residue after 60% ethanol extraction
Subfraction $XXII_5$	Water-soluble portion of residue after 60% ethanol extraction.

Baking Tests and Analytical Procedures

Experimental Baking Tests. To test the soy flour fractions, many of which were available only in small quantities, a modification of the small-scale baking test described by Geddes *et al.* (10,11), employing 25 g. of wheat flour, was used. The formula and procedure are summarized in Table I. Each mix yielded one 40 g. dough; replicates were baked on successive days. Loaf volumes were determined by an adaptation of the conventional seed displacement method to a special small-scale apparatus which was calibrated with dummy loaves.

The soy flour fractions were included in dough formulas to the extent of 3% (dry basis) of the weight of wheat flour (14% moisture basis). Water absorption was adjusted as required to give uniform consistency, as judged by handling properties.

TABLE I

BASIC FORMULA AND PROCEDURE — SMALL-SCALE LABORATORY BAKING TEST

	% OF WHEAT FLOUR (14% Moisture Basis)	WEIGHT
		g/mix
Flour[a]	100	25.0
Yeast	3	0.75
Sugar	5	1.25
Salt	2	0.5
Potassium bromate[b]	variable	....
Water[c]	60	15.0

Mixing time[d]	2.5 min.
Dough weight	40 g.
Fermentation[e] at 30°C., 90% R.H.	
Punched[f] after	95 min.
Molded after	25 min.
Proofing time	55 min.
Oven time[g] at 232°C.	15 min.

[a] Commercial Southwestern Bakers' Patent Flour, ash 0.43%, protein 11.9%, both expressed on a 14% moisture basis.

[b] Potassium bromate was included to the extent of 0, 2, 4, or 6 mg/100 g wheat flour.

[c] Proper absorption was established at 60% according to dough handling properties.

[d] Mixing was done with a 25-35 gram Micro Mixer, supplied by the National Mfg. Co., Lincoln, Nebraska. The mixing head was operated at 120 r.p.m.

[e] Carried out in a "Humi-Temp" fermentation cabinet.

[f] Punching was done in a National dough sheeter, with rolls modified to an effective width of 1.5 inches and set 3/16 inch apart. For panning, doughs were sheeted twice, at 3/16 and 1/8 inch roll settings. Molding was done on a National dough molder. Pan size: top 7.1 by 4.6 cm; bottom 6.0 by 3.6 cm; depth 3.2 cm.

[g] Baking was carried out in a National Rotary hearth-type oven.

Moisture, Ash, and Nitrogen Determinations. Moisture in soy flour fractions was in most cases determined by the forced-draft oven procedure, described in a previous paper (24). However, dialyzable fractions high in sugar were heated in a vacuum oven at 80°C. under less than 10 mm. pressure for 5 hours and the loss in weight determined. Nitrogen and ash determinations were made as previously described (24). For fractions available only in small quantities, nitrogen was determined on a semimicro scale using selenium oxychloride as a catalyst.

Determination of Inorganic Constituents. Qualitative and quantitative tests for ions were applied to fractions containing appreciable inorganic material. Cations were determined qualitatively by semimicro methods after ashing (5).

Chloride was determined quantitatively in certain fractions as a measure of the quantity of sodium chloride derived from pH adjustments with acid and alkali during fractionation. The chloride was precipitated with excess standard silver nitrate, the organic matter destroyed with potassium permanganate, and the residual silver nitrate titrated with standard potassium thiocyanate solution using saturated ferric alum solution as an indicator.

A procedure was devised for the determination of zinc, calcium, magnesium, and phosphate on a single sample. Soy flour fractions were "wet-ashed" by digesting with concentrated sulfuric and nitric acids and hydrogen peroxide as prescribed by Sullivan and Near (27). Zinc was determined gravimetrically by precipitation as the sulfide, ignition and weighing as zinc oxide according to Furman (9). Phosphate was assayed in the filtrates by precipitation as the molybdate, ignition and weighing as $P_2O_5 \cdot 24MoO_3$ (15,23).

Prior to the analysis for calcium, excess molybdate reagent was removed by the procedure of Furman (9). Calcium was precipitated as the oxalate, which was ignited and weighed as the oxide (15). Analysis for magnesium was then accomplished by destroying excess ammonium oxalate, precipitation as magnesium ammonium phosphate, followed by ignition and weighing as magnesium pyrophosphate (23).

Sulfhydryl Groups. Sulfhydryl groups were determined qualitatively by the nitroprusside test and quantitatively by the amperometric method of Larson and Jenness (17).

Chromatographic Methods. Sugars were determined qualitatively and quantitatively in soy flour fractions derived from the dialysate fraction of soy flour using the chromatographic methods described by Koch *et al.* (14).

For quantitative sugar estimation, unknown solutions were applied

to the paper in standard aliquots; guide strips along the sides of the chromatograms containing known sugars were removed, developed, and used to locate the individual sugars resolved from the test solution originally placed on the chromatogram. By reference to the guide strips, areas of the chromatogram containing the individual sugars were cut off, and the sugars were extracted by 15-minute treatment with a measured volume of water. The sugars were then determined spectrophotometrically by the phenol-sulfuric acid method of Dubois *et al.* (6).

Determination of Antitryptic Activity. Fraction XVIII, the nondialyzable fraction of the supernatant after protein precipitation, was obtained in a manner quite similar to the method employed by Klose *et al.* (13) in preparing a trypsin inhibitor fraction. The antitryptic activity of fraction XVIII was therefore of interest, and was determined by the method of Borchers *et al.* (2).

Determination of Dough pH. A Coleman Model 3 C pH electrometer was adapted to measure the pH of 1- to 2-g. dough portions. The measurement was accomplished by inserting the dough piece in the 5-ml. cup containing a few drops of potassium chloride solution; contact with the calomel electrode was established through a potassium chloride bridge. The glass electrode was inserted, the system allowed to equilibrate, and the pH determined in the conventional manner. Dough pH values thus obtained were in good agreement with values for larger dough samples and with those found in the literature (16).

Determination of Carbon Dioxide Production and Retention in Doughs. Production and retention of carbon dioxide in fermenting doughs which contained soy flour fractions and other adjuncts were measured with the Chopin Zymotachygraphe (4) at 30°C.

Doughs used for gas production and retention tests were made up of 125 g. Southwestern wheat flour (14% moisture basis) and other ingredients in the proportions given in Table I. Potassium bromate was included at a level of 2 mg. per 100 g. of flour; the various adjuncts were added at a level of 3% dry matter based upon flour at 14.0% moisture. Each dough was allowed to ferment for 6 hours.

Results

Results of Fractionation Procedure. The approximate percentage distribution of the fractions in defatted raw soy flour prepared as outlined in Fig. 1, together with their moisture and nitrogen contents are presented in Table II. Quantitative separation and recovery of all fractions was not practical; where feasible, losses were estimated, and the distribution figures are approximate only. The total weight of material

recovered included 1.5% of sodium chloride, which was calculated as having been derived from pH adjustments and distributed among several fractions.

The isolated protein (fraction XIII) after purification contained 15.7% nitrogen and was nearly white and essentially tasteless. However, it was rendered low in water dispersibility by the isolation treatment involving acid. Re-extraction of the water-insoluble material

TABLE II

MOISTURE AND NITROGEN CONTENTS AND APPROXIMATE PERCENTAGE DISTRIBUTION OF SOY FLOUR FRACTIONS

Fraction No.[a]	Fraction	Moisture Content "As Is" Basis	Nitrogen Content (Dry Basis)	Calculated % in Raw Defatted Soy Flour (Dry Basis)
		%	%	%
VI	Laboratory-defatted raw soy flour	8.7	9.12	100
IX	Fraction of water-insol. portion, insoluble at pH 8.5	7.6	3.14	21.1
X	Fraction of water-insol. portion, soluble at pH 8.5	6.2	12.82	13.9
XIII	Protein precipitated at pH 4.2 and dialyzed	6.0	15.70	30.4
XIV	Fraction of protein dialysate insoluble in 1:1 ethanol	11.0	11.78	0.3
XV	Fraction of protein dialysate soluble in 1:1 ethanol	8.4	3.82	0.9
XVII	Precipitate during dialysis of protein supernatant	6.0	13.62	0.7
XVIII	Nondialyzable fraction of protein supernatant	9.3	12.36	4.3
XX	Fraction of dialysate (of protein supernatant) insoluble in 1:1 ethanol	12.0	0.76	1.9
XXI	Crystals from hot ethanol extracts of dialysate	...	0.22	0.2
XXII	Precipitate upon addition of ethanol-insoluble portion of dialysate to methanol	10.2	1.27	9.6
XXIII	Crystals from methanolic supernatant	...	0.098	0.6
XXIV	Remainder of methanolic fraction[b]	25.7	1.28	6.0
XXV	Fraction of dialysate soluble in aqueous ethanol, insoluble in absolute ethanol[b]	28.2	2.36	7.2
XXVI	Precipitate upon addition of ethanol-soluble residue above to ethyl ether[b]	15.9	3.04	0.8
XXVII	Material soluble in ethyl ether	...	...	trace
	Total			97.9[c]

[a] See Fractionation Scheme, Fig. 1.
[b] Moisture content determined as loss of weight upon heating 6 hours at 80°C. and less than 10 mm. pressure.
[c] Includes 1.5% sodium chloride distributed among various fractions, calculated to have been derived from pH adjustments.

with very dilute alkali (pH 8.5) dispersed additional protein, as evidenced by the high nitrogen content of fraction X.

Baking Tests. The inclusion of 3% of raw soy flour in the baking formula significantly decreased loaf volume at lower bromate levels[4], as shown in Fig. 2. Color and flavor, as well as the grain and texture of the crumb, were also adversely affected. The experimental soy flour was inferior to a commercial unheated soy flour (II)[5] in general baking quality. Both showed a tendency to "buffer" dough against the injurious effect of excess potassium bromate.

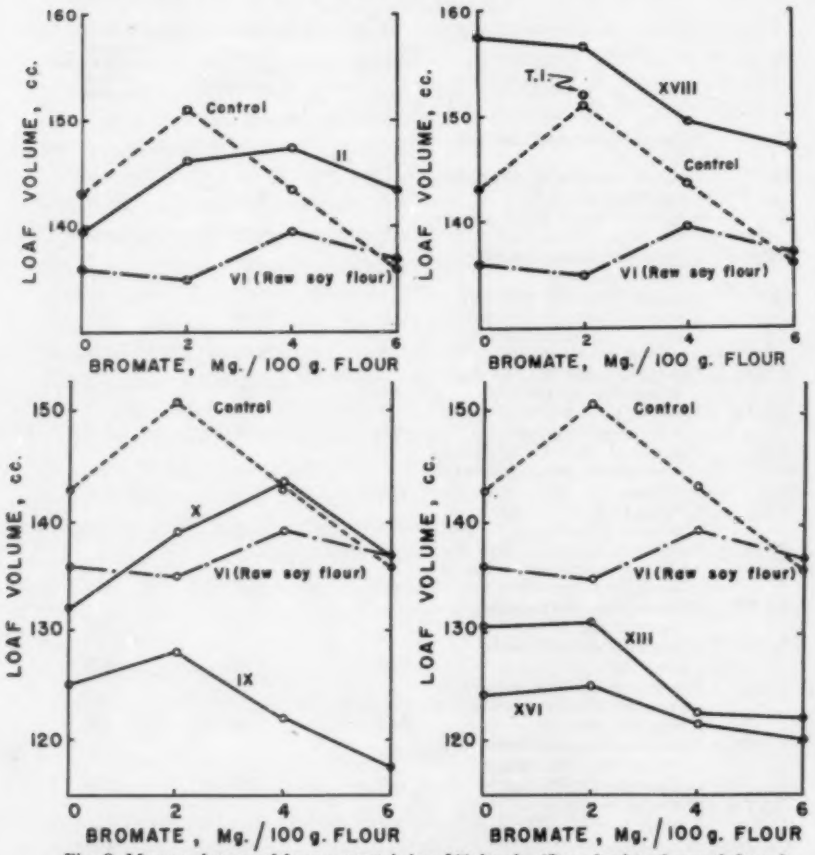

Fig. 2. Mean volumes of loaves containing 3% levels (flour basis) of raw defatted soy flour and several of its fractions in comparison with control (no soy flour).

[4] The standard error for a single loaf volume determination by the 40 g. dough procedure, as calculated from the largest series of bakes was 2.7 cc.

[5] Courtesy Archer-Daniels-Midland Co., Minneapolis, Minnesota.

Fraction XVIII (Fig. 2) was of very good baking quality, far excelling raw soy flour and definitely improving the control loaf. Also shown is the performance at one bromate level of a trypsin inhibitor (TI) prepared by an independent procedure. These results will be discussed later.

In the course of fractionation, it became clear that undesirable color and flavor principles as well as adverse loaf volume effects were concentrated in the dialysate fraction (No. XIX, Fig. 1). As the further fractionation of the dialysate progressed, it was possible to separate, to a large extent, components responsible for these effects. In Fig. 2 are plotted mean loaf volumes obtained from formulas containing 3% of fractions XX, XXII, XXIV, and XXV baked at four bromate levels. Fractions XX and XXII greatly decreased loaf volume and affected grain and texture adversely; No. XX had the most undesirable flavor. Fraction XXIV was highly colored and caused poor grain and texture but was superior in flavor to raw soy flour and nearly equal in volume

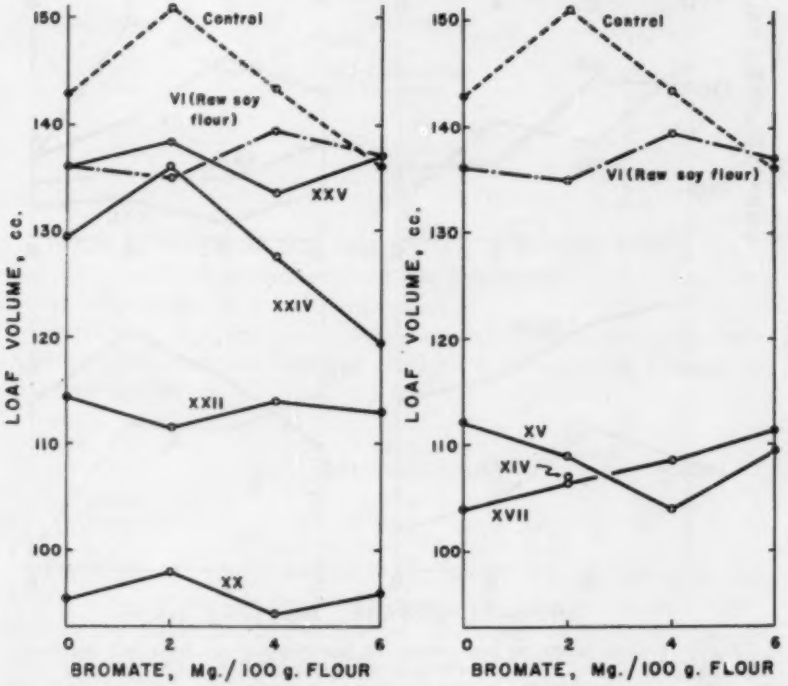

Fig. 3. Mean volumes of loaves containing 3% levels (flour basis) of raw defatted soy flour and several of its fractions in comparison with control (no soy flour).

potential. Fraction XXV affected chiefly flavor. Both XXIV and XXV tended to "buffer" against excess bromate.

The effects of three other injurious fractions, Nos. XIV, XV, and XVII, are shown in Fig. 2. Each was a minor fraction, constituting less than 1% of the raw soy flour. Fraction XVII is of interest as the only one which showed a positive response to potassium bromate.

Figure 3 depicts the performance of several fractions of medium baking quality. Fraction X (Fig. 3) was comparable with raw soy flour,

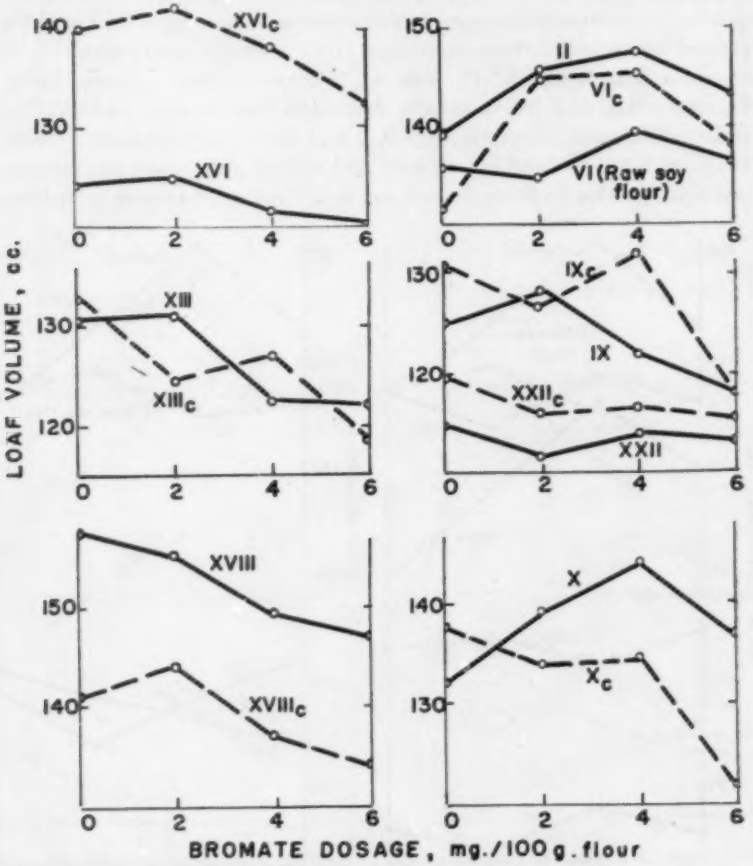

Fig. 4. Typical effects on loaf volume of heat-treating soy flour and soy flour fractions at 100°C. for 1 hour. Heat-treated fractions are designated by subscript "C."
Top row — fractions improved by heat treatment
Middle row — fractions not appreciably affected by heat treatment
Bottom row — fractions injured by heat treatment

while No. IX was lower in over-all quality. Both were superior to raw soy flour in flavor. Fractions XIII and XVI (Fig. 3), protein fractions prepared by different isolation techniques, were inferior in volume potential to raw soy flour but produced a better flavor.

The effects on the baking quality of raw soy flour and certain fractions, brought about by heat treatment for 1 hour at 100°C. and employing the moisture levels given in Table II, are shown in Fig. 4. Raw soy flour (VI) was improved by heat treatment (VI_c) so as to be similar to commercial soy flour II. A marked improvement in the baking quality of fraction XVI upon heat treatment (XVI_c) was noted; protein preparation XIII was virtually unaffected upon heating ($XIII_c$). Heat treatment proved injurious to fraction XVIII and to fraction X, both of which are high protein materials. None of the other fractions, including the most injurious in bread, was appreciably changed in baking quality by the conditions of heat treatment which were employed.

Chloride Content. The chloride contents of four fractions derived from the dialyzable material of soy flour are compared below with the general baking quality of these fractions:

Fraction No.	*Chloride Content as NaCl (dry basis)*	*Baking Quality*
	%	
XX	0.7	Poor
XXII	1.1	Poor
XXIV	14.4	Medium
XXV	16.5	Medium

It can be concluded that the presence of sodium chloride introduced through pH adjustments was not responsible for the poor baking quality of the most injurious fractions.

Antitryptic Activity. Antitryptic activities determined for fraction XVIII and for a trypsin inhibitor prepared as described by Klose *et al.* (13) were as follows:

	Nitrogen (dry basis)	*Inhibitor Units × 10³ per mg. (d.m. basis)*
Fraction XVIII	12.36	4.25
Trypsin inhibitor[9]	14.35	1.62

The high antitryptic activity of fraction XVIII suggests that the fractionation procedure up to this point was relatively mild. In the light of the researches of Learmonth (18,19) and of Melnick's patent (20), the marked improving effect of this fraction might perhaps be ascribed to its high antitryptic activity. However, there is no direct

[9] Preparation of this inhibitor and assay of antitryptic activity were carried out by Dr. I. E. Liener.

TABLE III
CONCENTRATIONS OF SUGARS IN SOY FLOUR FRACTIONS AND RAW SOY FLOUR

Sugar	Soy Flour Fraction				Raw Defatted Soy Flour Calculated[a]
	XX	XXII	XXIV	XV	
	%	%	%	%	%
Stachyose[b]	1.9	31.8	10.7	0.9	3.8
Raffinose	...	2.8	4.0	1.3	0.6
Sucrose	2.6	10.8	37.8	35.0	6.2
Galactose	...	1.5[c]	3.0	2.7	0.5
Glucose	...	...	2.9	2.3	0.3
Fructose	...	...	1.9	2.9	0.3

[a] Calculated from the percentage distribution figures in Table II upon the assumption that the fractions for which analyses are given contained all of the sugars present in the soy flour.
[b] Calculated with reference to standard curve for raffinose.
[c] Single determination. All others are means of two or more determinations.

evidence available that the trypsin inhibitor decreases the activity of the wheat flour proteinases on the flour proteins.

Sugars. The determination of sugars in several soy flour fractions aided in their characterization, although the sugars were not considered responsible for poor baking quality. The concentrations of the sugars are shown in Table III. The sugars of the original soy flour are also shown; they are calculated with the aid of the percentage distribution figures in Table II upon the assumption that the fractions for which analyses are given contained all of the sugars present in the soy flour. Large quantities of sugars (45–60%) were detected in fractions XXII, XXIV, and XXV, all obtained from the dialyzable fraction of raw soy flour. Of particular interest are the high percentages of stachyose in fraction XXII and of sucrose in Nos. XXIV and XXV. Values for stachyose are approximate, since they were calculated from the standard curve for raffinose. However, the estimated stachyose and sucrose contents for soy flour are in good agreement with the literature values (3, 25, 26). The raffinose content is somewhat lower than is given in the literature. Values for reducing sugars (galactose, glucose, fructose) are somewhat high: this may be due to some hydrolysis of sucrose and stachyose during isolation (12).

Sulfhydryl Groups. The possible presence of sulfhydryl groups in the soy flour fractions was of interest in view of the well-known adverse effect of certain compounds of this type in bread dough. The following data are representative of the range of sulfhydryl titer and of baking quality encountered among the fractions:

Fraction	*Sulfhydryl (as Cysteine)*	*Baking Quality*
	%	
XX	Negligible	Poor
XXII	Negligible	Poor
XXIII	0.066	Medium
XVI	0.082	Medium
XVIII	0.050	Good
Trypsin inhibitor	0.050	Good

These results do not reveal any relationship between baking performance of the soy flour fractions and their sulfhydryl contents.

Hydrogen Ion Activity. The pH of control doughs varied from 5.70 to 5.85 upon mixing; doughs containing soy flour fractions (3%) ranged in general from 5.28 to 5.98. An exception was fraction XX, the most injurious fraction in bread, which yielded a more alkaline dough. The following pH and loaf volume data are of interest in this connection (means of duplicate values; bromate 2 mg. per 100 g. flour):

	pH	*Mean Loaf Volume*
		cc
Control	5.70	151
VI raw soy flour	5.78	135
XX	6.20	98
XXII	5.90	112
Sodium hydroxide (0.32 meq.)	6.50	142

To determine whether the adverse effect of fraction XX might be attributed solely to abnormal pH, dilute sodium hydroxide solution (0.32 meq.) was added to the baking formula with the above results. Artificial adjustment of dough pH to an alkalinity in excess of that caused by fraction XX or XXII produced a far superior loaf so that the deleterious influence of these fractions cannot be traced to their effects on dough pH.

Further Study of Fractions XX and XXII. Fraction XX was only partially water-soluble, and it was separated into its water-insoluble (XX_1) and water-soluble (XX_2) components. A baking test using 2 mg. bromate per 100 g. flour was applied with the subfractions in amounts corresponding to the 3% level of No. XX, which gave the following results:

Adjunct	*Percent Based on Whole Fraction*	*Dry Matter Added*	*Loaf Volume*
		g	*cc*
Control	. . .	. .	144
XX_1 (water-insoluble)	26	0.19	148
XX_2 (water-soluble)	74	0.56	110
XX	100	0.75	98

The results demonstrated clearly that the water-soluble constituents of fraction XX were responsible for its adverse effects in baking.

The results of ion exchange subfractionation of fraction XXII and the baking tests are summariezed in Table IV. The distribution of nitrogen in the subfractions revealed that constituent(s) high in nitrogen were unrecovered. A portion of the unrecovered material is con-

TABLE IV

RECOVERY AND BAKING TESTS OF ION EXCHANGE SUBFRACTIONS OF NO. XXII

FRACTION	DRY MATTER DISTRIBUTION	NITROGEN CONTENT DRY BASIS	BAKING TESTS[a]		
			Level[b]	Dough pH	Loaf Vol.
	%	%	%		cc
Control	...	...	0	5.86	138
XXII	100.0	1.27	3	5.90	112
$XXII_a$ neutral	44.2	.02	3	5.96	124
$XXII_b$ ionic	47.4	.64	1.5[c]	4.80	88
Unrecovered	8.4	11.4[d]	...	...	...
Sucrose	...	...	3	5.78	128

[a] Means of duplicate values; bromate 2 mg/100 g flour.
[b] Level based on wheat flour at 14% moisture.
[c] Corresponds approximately to 3% level of No. XXII.
[d] Calculated.

sidered to be substances of basic character retained by the resins; the calculated average nitrogen value of 11.4% is a minimum value, since the unrecovered material also included losses of fractions $XXII_a$ and $XXII_b$ which were much lower in nitrogen.

Qualitative chromatograms demonstrated the presence in $XXII_a$ of the same sugars as were previously found in fraction XXII itself; subfraction $XXII_b$ was essentially devoid of sugars. Amino acids or other ninhydrin-positive substances were revealed in $XXII_b$ but not in $XXII_a$ (detected by spraying chromatograms with a solution of 0.4 g. ninhydrin and 10 g. phenol in 90 ml. of 70% ethanol)[7].

The ionic subfraction $XXII_b$ had a pronounced injurious effect in bread, although this might be due in part to abnormally low dough pH. The neutral subfraction ($XXII_a$), known to contain sugars in large amounts, had little influence on loaf volume; its effect was similar to that of a 3% excess of sucrose. These results point to the ionic character of the most harmful substances in fraction XXII.

Subfractions Obtained by Extraction of No. XXII with Ethanol-Water Solutions. The distribution of subfractions and results of baking tests are shown in Table V. Although the differences observed in baking quality among the subfractions were not great, the smallest volume and lowest grain and texture score resulted from the use of No. $XXII_5$. Dough pH was not a primary factor. The history of this subfraction is similar to that of No. XX_2, in which a deleterious principle was also shown to reside. The solubility relationships offer evidence of the ionic and inorganic character of the injurious substances.

The Role of Inorganic Constituents in the Performance of the Deleterious Fractions. Inorganic constituents were present in appreciable quantities in several fractions, as indicated by ash contents of 20–45%.

[7] These tests were made by Dr. J. E. DeVay, Department of Plant Pathology and Agricultural Botany.

On the basis of preliminary qualitative tests, quantitative determinations were carried out for zinc, magnesium, calcium, and phosphate in the most deleterious fractions. Results are shown in Table VI. Appreciable amounts of calcium, magnesium, and phosphate were found, especially in fraction XX. Although the quantities of zinc detected were quite small, zinc in small concentrations is known to have a marked effect on gas production and loaf volume (8).

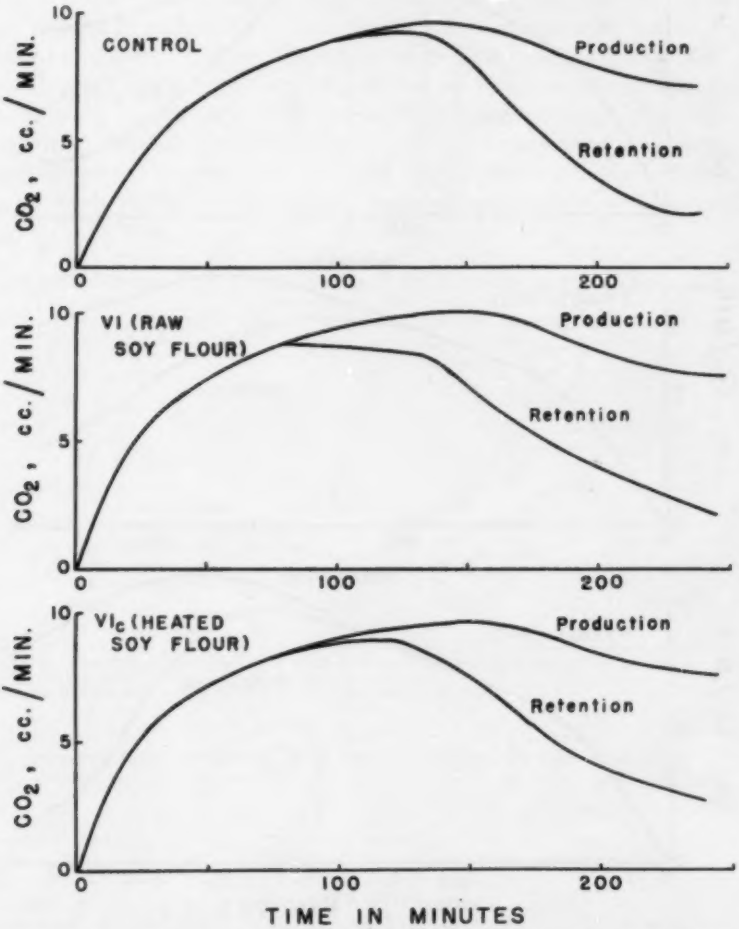

Fig. 5. Zymotachygraphe curves for doughs made without soy flour and with 3% of raw and heat-treated soy flour. Doughs were fermented at 30°C.

Since certain ions were detected in considerable quantity in the most injurious fractions, the effects of pure salts in bread were investigated. Cations were added as their chlorides, phosphate as a mixture of the monosodium and disodium salts; the level of each ion was selected to correspond to the amount found in the 3% level of fraction XX. The chlorides of sodium and potassium were included in these tests,

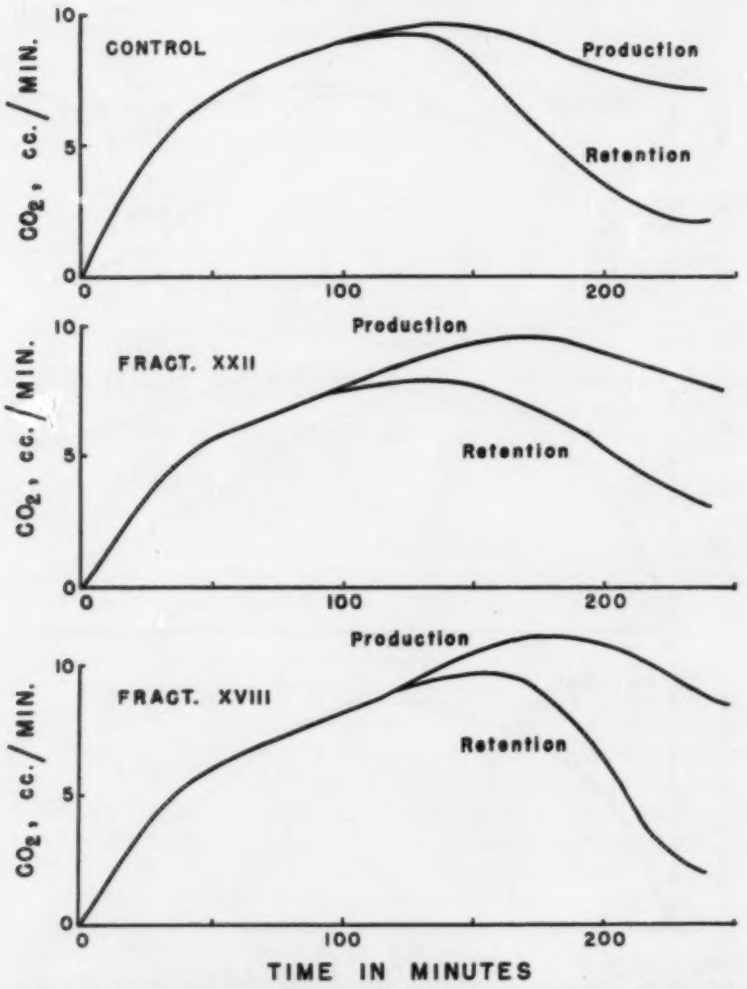

Fig. 6. Zymotachygraphe curves for doughs made without soy flour and with 3% additions of fractions XVIII and XXII. Doughs were fermented at 30°C.

although they were not determined quantitatively in the fractions. The levels of ions employed and the loaf volumes are presented in Table VII. Grain and texture scores were closely related to volumes, while flavor and color were not noticeably affected.

A 0.2% excess of sodium (as chloride) had no significant effect, whereas 1.0% excess was injurious. An equal weight of potassium (as chloride) was somewhat less injurious. Calcium and magnesium chlorides slightly increased loaf volume. A small quantity of zinc chloride profoundly affected bread quality, 0.0048% being sufficient to reduce loaf volume by nearly 20%. This result is in harmony with the observations of Finney *et al.* (8). Phosphate at the level used was also injurious to bread quality. Although the data do not indicate the individual effects of the ions, it seems certain that the zinc and the large phosphate content of the most injurious fractions were instrumental in causing poor volumes. However, in view of the adverse effects of other salts, the performance of these fractions must be attributed in part to a general

TABLE V

BAKING PERFORMANCE OF SUBFRACTIONS OF XXII OBTAINED BY EXTRACTION WITH ETHANOL-WATER MIXTURES[a]

	Adjunct	Distribution[b] (% of Fract. XXII, dry basis)	Nitrogen (dry basis)	Dough pH	Loaf Volume
			%		cc
Control			. . . .	5.79	148
XXII			1.27	5.90	112
$XXII_1$	80% ethanol extract	5.0	1.09	5.67	117[b]
$XXII_2$	70% ethanol extract	25.0	1.18	5.72	114[b]
$XXII_3$	60% ethanol extract	35.2	1.32	5.75	115
$XXII_4$	Water-insoluble portion of residue after 60% ethanol extraction	2.4	0.96	6.08[b]	120[b]
$XXII_5$	Water-soluble portion of same	37.1	1.10	6.02	106
	Total	101.7[c]			

[a] Adjuncts included at 3% level, based on wheat flour at 14% moisture. Bromate 2 mg/100 g flour.
[b] Single determination. Others are means of duplicate values.
[c] Total greater than 100% due to additive errors in moisture determination.

TABLE VI

LEVELS OF INORGANIC CONSTITUENTS FOUND IN SEVERAL SOY FLOUR FRACTIONS

Ion	Fraction		
	XX	XXII	$XXII_5$
	%	%	%
Zinc	0.16	0.06	0.00
Calcium	5.76	0.34	0.53
Magnesium	4.15	1.71[a]	2.74[a]
Phosphate, as PO_4	20.00	3.64	6.47

[a] Single determination. All others are means of duplicate determinations.

TABLE VII

INFLUENCE OF ADDED SALTS ON BREAD QUALITY[a]

Ion Employed	Quantity Added[b]	Loaf Volume[c]
	%	cc
Control, none	...	144
Sodium	0.20	142
Sodium	1.00	128
Potassium	1.00	137
Calcium	0.172	152
Magnesium	0.124	156
Zinc	0.0048	118
Phosphate (as monobasic sodium salt)	0.30	120
Phosphate (as dibasic sodium salt)	0.30	

[a] Bromate 2 mg/100 g flour.
[b] Based on weight of wheat flour. Included in addition to normal salt content of formula (2%).
[c] Means of duplicate values.

salt effect, in addition to specific effects of ions shown to be particularly harmful.

Effect of Soy Flour Fractions and Inorganic Ions on Gas Production and Retention during Fermentation. Figures 5, 6, and 7 show curves obtained by plotting against fermentation time the maximum gas production or retention recorded by the zymotachygraphe during each 10-minute cycle. In relating the curves to baking tests, it should be recalled that the fermentation period was 2.0 hours, followed by a 55-minute proofing period. The fact that the baking test involved manipulation of the dough at two stages (thus altering its gas-retaining properties) whereas the zymotachygraphe tests involved no such treatment, dictates caution in interpreting the curves.

Raw soy flour itself (Fig. 5) slightly increased gas production during the entire fermentation period but caused retention to fall off after about 1.5 hours. Heated soy flour (VI_c), although it hampered gas production, improved retention and was also superior in baking quality to the raw soy flour (Fig. 4, upper right). The curves indicate that gas retention is the limiting factor in these baking tests. Fraction XIII, a protein fraction for which no curve is shown, was very similar to raw soy flour in its effects on production and retention.

Fraction XXII, poor in baking quality, lowered both the production and retention of carbon dioxide (Fig. 6). The poor baking quality may be due to the decline in retention early (about 1.5 hours) in the fermentation period.

Fraction XVIII, which was of excellent baking quality, lowered gas production during the early part of the fermentation, but both production and retention were augmented during the critical proofing period (third hour).

As shown in Fig. 7, the inclusion of zinc ion, or phosphate, each in

the amount corresponding to a 3% level of fraction XX, seriously retarded gas production. Under these conditions, the retention curve has little meaning. Although zinc and phosphate ions seemed to be important in the performance of the fractions of poorest baking quality, they influenced primarily gas production; soy flour and soy flour protein without oxidation or heat treatment affected chiefly gas retention. Thus, the inorganic constituents would seem to be of secondary importance in the over-all performance of soy flour.

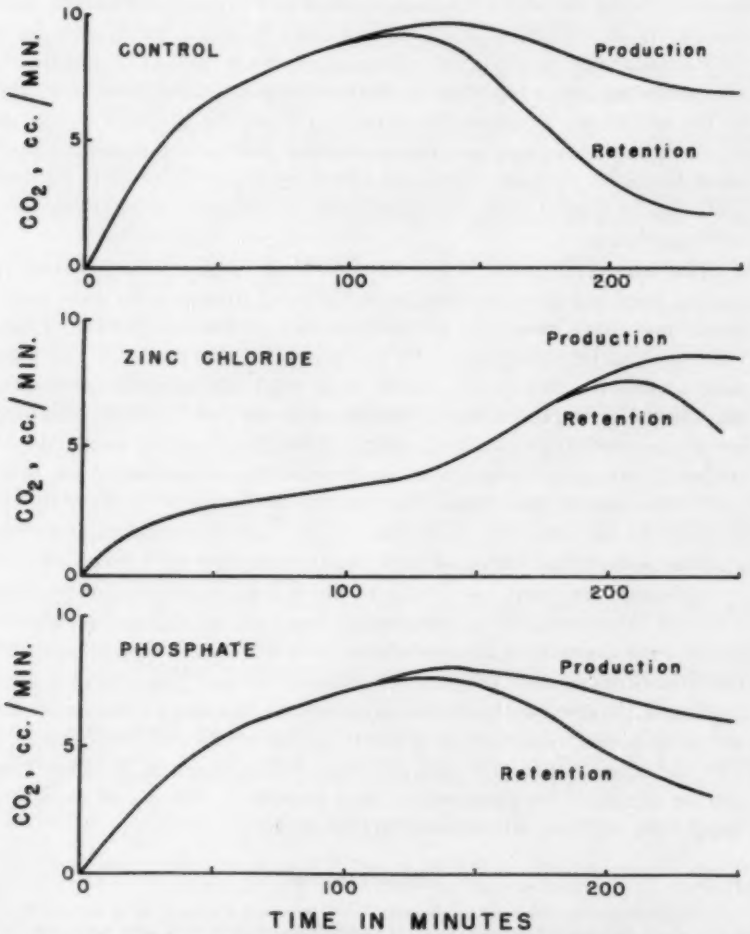

Fig. 7. Zymotachygraphe curves showing the influence of 0.0048% zinc ions and 0.60% phosphate ions (expressed on flour basis) on gas production and retention.

Discussion

The chief endeavor in this study was to obtain reproducible fractions from soy flour, to determine their influence on breadmaking, and to relate their chemical composition with baking performance. In the baking tests, the soy flour fractions were employed at a level of 3% (flour basis), without regard to the relative proportions present in the soy flour. In this way, the various fractions were employed at sufficiently high levels to secure definitive results which were influenced only by the nature of the fraction. It must be recognized, however, that the effects of individual fractions obtained in small yields may have little significance in the performance of soy flour itself. The possibility also exists that the properties of the fractions may have been modified by the techniques employed in obtaining them. An attempt was made to relate the baking qualities of recombined fractions to those of equivalent fractions. In many cases, the effects on loaf volume were equivalent, but, in several cases, the differences in loaf volume were statistically significant.

The beneficial effect of 1% raw soy flour, which was observed in baking tests at a low bromate level (24) and destroyed by heat treatment, may have been due to the presence of fraction XVIII, which was found to be very heat-labile. No beneficial effects of raw soy flour were observed when 3–5% levels were used (24) without increasing the oxidizing improver level. Baking tests on the fractions obtained in the present study revealed widely different effects of heat. At the higher bromate levels employed in these tests, raw, extracted soy flour itself was improved by heat. The baking quality of soy flour thus appears to be the resultant of the baking quality of individual fractions and the differential effects of heat and bromate level.

Although the restricted nature of the baking tests does not warrant a broad interpretation of the results, it would appear to be difficult to improve the baking quality of soy flour by the removal of any specific constituents, since major components were as injurious as soy flour itself and adverse effects on loaf volume and flavor were traced to different fractions. However, as pointed out by several other workers (1, 7,21,22), there is promise that soy flour performance in breadmaking can be enhanced by appropriate heat treatment, the use of oxidizing improvers, and the adjustment of fermentation schedules.

Acknowledgments

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Literature Cited

1. Bayfield, E. G., and Swanson, E. C. Effect of yeast, bromate and fermentation on bread containing soy flour. Cereal Chem. **23**: 104–113 (1946).
2. Borchers, R., Ackerson, C. W., and Sandstedt, R. M. Trypsin inhibitor. III. Determination and heat destruction of the trypsin inhibitor of soybeans. Arch. Biochem. **12**: 367–374 (1947).
3. Burrell, R. C., and Wolfe, A. C. A comparative study of the chemical composition of five varieties of soybeans. Food Research **5**: 109–113 (1940).
4. Chopin, M. Le Zymotachygraphe. Appareil de controle de la fermentation panaire. Bull. anciens élèves école franc. meunerie **134**: 70 (1953).
5. Dobbins, J. T. Semi-micro qualitative analysis. Wiley: New York (1943).
6. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**: 350–356 (1956).
7. Finney, K. F. Loaf volume potentialities, buffering capacity, and other baking properties of soy flour in blends with spring wheat flour. Cereal Chem. **23**: 96–104 (1946).
8. Finney, K. F., McCammon, J. F., and Schrenk, W. C. Effect of varying concentrations of certain metals and their salts on gas production and loaf volume. Cereal Chem. **26**: 140–148 (1949).
9. Furman, N. H., ed. Scott's standard methods of analysis (5th ed.). D. van Nostrand: New York (1939).
10. Geddes, W. F., and Aitken, T. R. An experimental milling and baking technique requiring 100 grams wheat. Cereal Chem. **12**: 696–707 (1935).
11. Geddes, W. F., and Sibbitt, L. D. Variability in experimental baking. IV. Studies on mixing, sheeting rolls, pan shape and 50 and 25 gram formulas. Cereal Chem. **10**: 560–584 (1933).
12. Gortner, R. A. Outlines of biochemistry (3rd ed.). Chap. 14. Wiley: New York (1949).
13. Klose, A. A., Hill, Barbara, and Fevold, H. L. Presence of a growth inhibiting substance in raw soybeans. Proc. Soc. Exptl. Biol. Med. **62**: 10–12 (1946).
14. Koch, R. B., Geddes, W. F., and Smith, F. The carbohydrates of Gramineae. I. The sugars of the flour of wheat (Triticum vulgare). Cereal Chem. **28**: 424–430 (1951).
15. Kolthoff, I. M., and Sandell, E. B. Textbook of quantitative inorganic analysis (2nd ed.). Macmillan: New York (1947).
16. Lanning, J. H. The effect of sucrose and maltose upon acid and gas production in doughs. Cereal Chem. **13**: 690–697 (1936).
17. Larson, B. L., and Jenness, R. The determination of protein sulfhydryl groups with iodine and *o*-iodosobenzoate by an amperometric titration. J. Dairy Sci. **33**: 890–895 (1950).
18. Learmonth, E. M. The influence of soya flour on bread doughs. I. A papain-inhibiting factor in soy beans. J. Sci. Food Agri. **2**: 447–449 (1951).
19. Learmonth, E. M. The influence of soya flour on bread duoghs. II. The inhibition of the proteolytic enzymes of malt and wheat flours. J. Sci. Food Agri. **3**: 54–59 (1952).
20. Melnick, D. Inhibiting protein degradation in food. U. S. Patent 2,670,291. Feb. 23, 1954 (Chem. Abstracts **48**: 6048b).
21. Ofelt, C. W., Smith, A. K., and Derges, R. E. Baking behavior and oxidation requirements of soy flour. I. Commercial full-fat soy flours. Cereal Chem. **31**: 15–22 (1954).
22. Ofelt, C. W., Smith, A. K., and Mills, J. M. Baking behavior and oxidation requirements of soy flour. II. Commercial defatted soy flours. Cereal Chem. **31**: 23–28 (1954).

23. PIERCE, W. C., and HAENISCH, E. L. Quantitative analysis (2nd ed.). Wiley: New York (1940).

24. POLLOCK, J. M., and GEDDES, W. F. Soy flour as a white bread ingredient. I. Preparation of raw and heat-treated soy flours and their effects on dough and bread. Cereal Chem. 37:19–29 (1960).

25. SATOW, S. Researches on oil and proteids extraction from soybean. Tech. Repts. Tohoku Imp. Univ. 2: 41–125 (1921).

26. STREET, J. P., and BAILEY, E. M. The carbohydrates and the enzymes of the soybean. Ind. Eng. Chem. 7: 853-858 (1915).

27. SULLIVAN, BETTY, and NEAR, CLEO. The ash of hard spring wheat and its products. Ind. Eng. Chem. 19: 498–501 (1927).

SOME VOLATILE AROMATIC COMPOUNDS IN FRESH BREAD[1]

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ABSTRACT

Volatile compounds in freshly baked bread are recovered with minimal decomposition by vacuum pumping from a steam-jacketed vessel containing the coarsely shredded bread. Efficient traps are necessary to condense the more volatile materials. The total condensate is treated to remove organic acids, and then the carbonyl compounds are converted to 2,4-dinitrophenylhydrazones (DNPH's). Separate aliquots are used to investigate alcohols and esters. No qualitative evidence has been found for the presence of phenolic compounds or amines.

The DNPH's have been separated by repeated adsorption chromatography on activated silica gel columns and, to a lesser degree, by paper chromatography. The aldehydes identified in this way are acetaldehyde, crotonaldehyde, 2-ethylhexanal, and furfural; the ketones are acetone, hexanone-2, heptanone-3; dicarbonyl compounds are diacetyl and methylglyoxal; pyruvic and levulinic acids are also present, possibly in the form of their ethyl esters. Several other DNPH's have not been identified positively.

Quantitative estimations have been made of some of the volatile compounds, and the data used in attempts to impart a typical breadlike flavor to a very bland, chemically leavened product. These attempts were unsuccessful, whether the treatment was applied to the dough ingredients or (by aeration) to slices of the freshly baked product.

The experiments of Baker and associates (1,2) have lent weight to the view that the flavor of fresh bread is due to a combination of the enzymatic and chemical reactions during fermentation of the dough and the thermal changes occurring in the oven, particularly associated with the formation of a brown crust. These workers believe that the more volatile compounds originally in the crust are drawn into the crumb during cooling of bread, where they are transformed by oxidative or other changes to substances imparting a "stale" flavor.

Most of the published information on the chemistry of bread flavor has been concerned with products other than the white pan bread most popular in America, notably rye breads. In view of the gross differences in flavor between these two classes of bread, any inferences drawn from the studies of European workers (12,15,19) on rye breads must be of limited application to white pan bread. Apart from the previously

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cited work of Baker (1,2), the meager published material on flavor substances in white bread includes the report of Visser't Hooft and deLeeuw (20), one of the first attempts to correlate a specific product of fermentation with the palatability of the bread.

In the work to be described, analytical methods which have yielded excellent results in other areas of food flavor research have been applied to those substances which exert significant vapor pressures in fresh bread. No attempt has been made to decompose nonvolatile complexes which might release volatile substances by chemical treatment (as, for example, splitting out organic acids from their salts or protein complexes). The objective has been to determine what compounds contribute directly to the aroma component of fresh flavor and in what concentration these compounds occur. A further objective, contingent upon the validity of the analytical results achieved, was to attempt the synthesis of a flavoring composition which would impart breadlike flavor to a very bland, chemically leavened product (14).

Materials and Methods

Bread Formula. In order to simplify the investigation as far as possible, only those ingredients absolutely essential to proper baking performance and crust formation were included. Such ingredients as milk solids and malt preparations were deliberately omitted, since they can contribute pronounced flavors of their own. The formula used was as follows (percentages are based on flour weight):

Flour (bakers patent from S.W. spring wheat)	100.0%
Water — as needed, usually about	64.0%
Yeast	2.5%
Sucrose	5.0%
Salt	2.0%
Lard	1.2%

Doughs were no-punch straight doughs, fermented about 3 hours at 24°C. They were scaled at 18.5 oz. and molded; proofed to height at 35°C.; and the loaves were baked at 232°C. (450°F.) for 25 minutes.

Recovery of Volatiles. Initial attempts to isolate volatile aromatic substances were based on steam distillation either of aqueous slurries of fresh bread or of filtrates from such slurries. In either procedure, there was evidence of severe breakdown during steam distillation with the production of foul-smelling artifacts, notably indole. The breakdown products were not as prominent when filtrates were steam-distilled, but some of the volatile aromatics are very poorly water-soluble and were not present in filtrates.

Attempts to omit the distillation step were unsuccessful. Whether

water or any organic solvent was used as the extractant, the extract contained relatively great amounts of sugars, lipids, etc., which reacted with any reagent used to derivatize the volatiles and made any subsequent separations extremely ineffective. Even when ether, benzene, or heptane extracts were dried over sodium sulfate and then distilled under vacuum, decomposition became excessive long before the bulk of the aromatics could be separated.

The most successful technique was to shred the freshly baked bread coarsely and to distill the aromatics (together with moisture) under high vacuum, supplying only sufficient heat to the crumb to maintain room temperature or slightly above. The apparatus comprised an aluminum pressure canner, modified so that the sealing gasket would seal under vacuum rather than pressure and with the pressure gauge and safety valve removed. An aluminum gooseneck vapor-takeoff was fitted to the lid with neoprene sealing gaskets. The canner was set into a steam jacket made from a 30-gallon steel drum to permit heating the charge during distillation. The vapors were led through a train of traps beginning with an ice bath and followed by at least two dry ice-alcohol traps. A mechanical vacuum pump was used as the vacuum source, and, when necessary, it was supplemented by an oil diffusion pump operating with a silicone oil (DC 703). The combination was capable of maintaining as low as 1 micron absolute pressure in the system, but normally such high vacuum was not necessary.

In operating this system, a relatively moderate vacuum (15–30 mm. Hg) was applied until nearly all the moisture had been removed from the bread. Usually, the bread was then pulverized to improve the transfer of heat from the container walls, and pumping was continued until the absolute pressure dropped below 50 microns measured with a McLeod gauge. After about 1 hour in this range, the residual bread was always free of any discernible odor, even if a sample were stirred with boiling water. If allowed to stand exposed to air, the dry residue rapidly developed a rancid odor, however.

Treatment of Recovered Volatiles. Of primary interest in these studies were the volatile carbonyl compounds. Regardless of whether these were separated from bread extracts or by direct vacuum distillation, as just described, it was considered important to convert carbonyl compounds into relatively stable derivatives at the earliest moment to minimize their decomposition. The 2,4-dinitrophenylhydrazones offer many advantages; they are formed readily and in almost quantitative yields; they can be separated from one another by chromatographic methods; and the identities of the parent carbonyl compounds can be

learned from melting-point and crystallographic data on the purified derivatives.

Generally, the 2,4-dinitrophenylhydrazones (DNPH's) were formed by adding an excess of 2,4-dinitrophenylhydrazine reagent in aqueous hydrochloric acid to the mixture containing the carbonyl compounds, so that the final mixture would be about 2*N* in acid. If the carbonyls were in an organic solvent, this was removed by evaporation *after* forming of the DNPH's. The latter were allowed to form overnight at room temperature and were filtered off on a coarse fritted-glass filter. After the solids were washed with warm 2*N* hydrochloric acid and water, they were dried thoroughly over sodium hydroxide pellets in a desiccator.

In the investigation of volatile acids, the vacuum distillate from fresh bread was titrated with sodium hydroxide until just alkaline, and then it was evaporated to dryness under reduced pressure using a rotary flash evaporator. The sodium salts were dissolved in a little water, acidified with a few drops of sulfuric acid, the solution was saturated with solid sodium sulfate, and the free acids were extracted with benzene. The benzene solution of the organic acids was used to spot chromatograms on Whatman No. 1 paper while the paper was exposed to ethylamine vapor. The chromatograms were also spotted with known organic acids (acetic, propionic, butyric, isobutyric, isovaleric, and caproic) and were developed with *n*-butanol:water:ethylamine (85:15:1), as described by Hiscox and Berridge (3).

No attempt was made to characterize volatile esters, since qualitative tests with hydroxylamine and ferric ion were negative. This suggested that any study of volatile esters would require amounts of bread too great to be handled successfully with the equipment at hand.

To study the alcohols in the bread volatiles, the vacuum distillate was first titrated to convert free acids to salts; it was then distilled at atmospheric pressure until the undistilled portion was odorless. This new distillate was next treated to form the DNPH's of the carbonyl compounds; the latter were filtered off and the filtrate was made approximately neutral and distilled at atmospheric pressure. This final distillate was treated according to the procedure of Holley and Holley (4); a portion was reserved for examination by gas chromatography.

Chromatography of DNPH's. Column adsorption chromatography was considered preferable to paper chromatography for the identification of individual carbonyl compounds via their DNPH's, since it is possible to isolate amounts sufficient to obtain mixed melting point data. The simplicity and sensitivity of paper chromatography made it a useful adjunct, however. Numerous adsorbents and solvent systems

were tried for column separations, among which may be mentioned alumina (17); magnesium sulfate (16); talc (18); and silicic acid (13). Silicic acid proved to be the most satisfactory adsorbent, when the columns were prepared as follows:

Silicic acid, reagent grade, 100 mesh ("suitable for chromatography") is dried for at least 8 hours at 180°C. and is stored in completely filled, screw-capped jars. To pack a column, the prepared adsorbent is cautiously slurried with anhydrous diethyl ether at the rate of 75 cc. ether per 20 g. of silicic acid (caution – heat is evolved). The slurry is poured into the tube, fitted with a porous retaining plate, and is compacted with 1 p.s.i. of air pressure. When the solvent level has almost reached the top of the packing, another 25 cc. of anhydrous diethyl ether are added, and the solvent is again forced down to the level of the packing. Then the diethyl ether is displaced by petroleum ether (boiling range 30°–60°C.), using a total of 150 cc. to ensure complete displacement of the diethyl ether. The column is now ready for application of the sample.

It is customary to apply pressure when operating these columns to increase the solvent flow rate. Experience in this laboratory indicated that solvent flow rates much lower than those usually specified (e.g., about 0.5 cc. per minute for a column 19 by 250 mm.) gave much cleaner separations of complex mixtures with improved homogeneity of the bands and freedom from streaking and channeling. Such flow rates could be attained by gravity flow alone, while the time to complete a run could be lessened safely by increasing the solvent system polarity more rapidly than was tolerable when air pressure was used.

Details of the adsorption chromatography have been omitted here; in no instance was it possible to isolate pure DNPH's from a mixture by passage through a single column. Large columns, about 42 mm. inside diameter, were used to achieve rough separations into groups, which in turn were applied to smaller columns and resolved further. As many as four separate columns might be used to purify some of the DNPH's.

Paper chromatography was helpful in examining the eluates from columns for homogeneity and usually indicated when a DNPH was sufficiently pure to be recrystallized for melting-point determinations. The solvent systems employed were mainly the *n*-heptane:methanol system of Huelin (6) and the decahydronaphthalene:dimethylformamide system of Horner and Kirmse (5). The DNPH's of the keto acids, and a few others which moved very slightly in the above systems, were handled preferably with the system *n*-butanol:ethanol:water (4:1:5). Examination of the developed chromatograms was facilitated by the

use of a viewing box containing "black light" fluorescent tubes, covered by two sheets of ground glass with a dark blue gelatin optical filter between. As viewed by the ultraviolet light transmitted from beneath the paper sheets, the spots appeared almost black against a pale blue background. In some cases, the sheets were sprayed with dilute alcoholic sodium hydroxide, which rendered the DNPH spots red, brown, or blue (the blue color indicating a bis-DNPH of a dicarbonyl compound).

Reference Derivatives of Known Carbonyl Compounds. In order to identify the DNPH's isolated from the volatile carbonyl compounds of bread, mixed melting points and chromatographic comparisons were made with authentic DNPH's of known carbonyl compounds. In general, the latter were prepared and purified by standard methods from commercially procured carbonyl compounds; the starting compounds were redistilled when there seemed any doubt of their purity (e.g., furfural). Melting points of the reference DNPH's were checked against those reported in Huntress (7), Johnson (10), Strain (18), and Jones *et al.* (11). Ultraviolet absorption spectra, taken with a Beckman DU Spectrophotometer, were also compared with those reported in the literature where available.

Quantitative Analytical Procedures. Volatile acids were estimated by conventional titration in aqueous medium. Ethanol was estimated by dichromate oxidation, neglecting any errors introduced by other oxidizable compounds. (The concentration of ethanol in the volatiles is greatly in excess of all other compounds detected.) Furfural was estimated by its ultraviolet absorption at 277 $m\mu$ after ascertaining with aqueous standard solutions that its molar absorbancy of 14,000 at this wave length is about 1000 times greater than the absorbancy of any other carbonyl compound identified. Crotonaldehyde, which exhibits a similarly strong absorption at 223 $m\mu$, did not interfere at 277 $m\mu$.

Total alpha-beta dicarbonyls were estimated, under the assumption that no acetoin or dihydroxy compounds were present in the volatiles. Acetoin, if present in the bread, has a low volatility, while glycols would be even less volatile. Periodic acid oxidation was therefore assumed to account only for diacetyl plus methylglyoxal, the two dicarbonyl compounds identified in the volatiles. The method followed is described by Jackson (8).

Diacetyl was estimated colorimetrically by its reaction with creatine and alkaline alpha-naphthol (21). It was determined first that, under the specified reaction conditions, methylglyoxal in equivalent amounts produced no measurable color. Methylglyoxal was then estimated by

the difference between total dicarbonyls and diacetyl.

It was not possible to estimate total carbonyls with any confidence. The usual volumetric methods based on oximation could not be applied successfully on the micro scale dictated by the small amounts of volatiles which were obtainable. Numerous methods based on measurement of the color developed by DNPH's in alcoholic alkali have been reported, but a careful survey of such methods in this laboratory[3] showed that they are uniformly incapable of yielding accurate results with known mixtures of carbonyl compounds and are useful only in comparative studies.

Supplementation of Chemically Leavened Bread. Before it could be assumed that any mixture of substances simulating the chemical composition of bread flavor might be useful as an additive to chemically leavened doughs, it was felt advisable to try an actual distillate from conventional white bread of good flavor. Sixteen loaves, baked from the formula already given with the addition of 4% nonfat dry milk, were stripped under vacuum, and the condensed volatiles were redistilled at atmospheric pressure. The original strippings, as was usual by the method employed, had an odor best described as "rubbery"; following the atmospheric distillation, the condensate had a more breadlike aroma. A sevenfold concentration of the flavorous material was thus obtained in the first 13.5% of distillate; the undistilled portion was essentially odorless. The concentrated distillate (360 ml.) was used as part of the dough water to make up a chemically leavened dough (14) based on 750 g. of flour. Two loaves were baked from this dough, along with two loaves from an unsupplemented dough.

A second approach was to expose slices of freshly baked, chemically leavened bread, in a vacuum desiccator, to the vapor from a bread distillate or from a synthetic "flavor" mixture. Such treatment would, it was felt, obviate losses and alteration of the volatiles through baking. The general procedure consisted of evacuating the desiccator with its contained slices to about 20 mm. absolute pressure and connecting the desiccator to a small vacuum oven containing the aromatic substances in a dish, previously evacuated to about ½ atmosphere. As the oven-venting cock was opened, the system slowly came to equilibrium at about 200 mm. absolute; the oven was being heated during this time up to a maximum of perhaps 90°C. Then nitrogen from a cylinder was allowed to bleed slowly into the oven and thence into the desiccator, sweeping volatiles into the latter. When the system reached atmospheric pressure, the desiccator was opened, and the slices were

[3] Kohn, F. E. Unpublished data. American Institute of Baking, 1957.

sampled. They had acquired a pronounced aroma in every instance.

Results and Discussion

Identification of Carbonyl Compounds. When converting the carbonyl compounds of fresh bread volatiles to the DNPH's, an amount of 2,4-dinitrophenylhydrazine reagent (in 2*N* hydrochloric acid) sufficient to ensure a considerable unreacted excess was always used. As keto-acid hydrazones are somewhat soluble in aqueous media, the filtrate from a derivative mixture was normally extracted with ethyl acetate to recover any keto-acid DNPH's, and these were re-extracted into dilute sodium bicarbonate solution, returning the ethyl acetate-soluble neutral DNPH's, if any, to the main bulk of precipitated derivatives.

Paper chromatography of the keto-acid DNPH's revealed two spots, whose R_f values corresponded to those of pyruvic acid DNPH and levulinic acid DNPH. By streaking the mixture on large sheets of Whatman 3MM paper, it was possible to resolve and recover sufficient of the two hydrazones to obtain mixed melting points with authentic samples of the supposed compounds and thereby to confirm their identities.

Pyruvic Acid DNPH. Pale yellow needles from hot water, m.p. 218°C. Mixed m.p. with authentic sample, 219°C.; m.p. of authentic sample, 220°C.

Levulinic Acid DNPH. Orange crystals from hot water, m.p. 206°C. Mixed m.p. with authentic sample, 206°C.; m.p. of authentic sample, 206°C.

The latter keto acid was found in the form of its ethyl ester by chromatographic isolation of the DNPH.

Ethyl Levulinate DNPH. Orange-yellow crystals from ethanol, m.p. 102°C. Mixed m.p. with authentic sample, 102°C.; m.p. of authentic sample, 103°C.

Among the 2,4-dinitrophenylhydrazones of the neutral carbonyl compounds, the most easily purified were those of the saturated aliphatic aldehydes and ketones, which are eluted from silicic acid adsorption columns with small concentrations of diethyl ether in petroleum ether.

2-Ethylhexanal DNPH. Orange-yellow needles from cold methanol and water, m.p. 120°C. Mixed m.p. with authentic sample, 122°C.; m.p. of authentic sample, 124°C.

Acetone DNPH. Orange-yellow needles from methanol, m.p. 127°C. Mixed m.p. with authentic sample, 126°C.; m.p. of authentic sample, 127°C.

Hexanone-2 DNPH. Orange plates from cold methanol and water, m.p. 100°–101°C. Mixed m.p. with authentic sample, 103°C.; m.p. of authentic sample, 104°C.

Heptanone-3 DNPH. Yellow needles from petroleum ether (−29°C.), m.p. 79°C. Mixed m.p. with authentic sample, 80°–82°C.; m.p. of authentic sample, 81°C.

The DNPH of acetaldehyde was always detectable on paper chromatograms, but this compound could not be resolved on adsorption columns. Orange needles which crystallized readily and melted at 134°–137°C. were obtained regularly and could not be identified until a paper chromatogram of these needles revealed two spots corresponding to the DNPH's of acetaldehyde and crotonaldehyde. Rapid heating of the orange needles to 170°C. caused vigorous ebullition, and fine yellow needles were obtained as a sublimate. These proved to be acetaldehyde DNPH. The melt resolidified after driving off this compound, and the brilliant red residue was shown to be crotonaldehyde DNPH. These two derivatives appear to form a complex of rather definite structure during desorption from silicic acid, while a certain amount of crotonaldehyde DNPH could be eluted in a pure state.

Acetaldehyde DNPH. Sublimate of fine yellow needles, m.p. 157°C. Mixed m.p. with resublimed authentic sample, 157°C. M.p. of sublimed (metastable form) authentic sample, 157°C.

Crotonaldehyde DNPH. Brilliant red crystals from ethyl acetate and petroleum ether, m.p. 193°–196°C. Mixed m.p. with authentic sample, 193°–195°C. M.p. of authentic sample, 190°–192°C.

The most difficult carbonyl compounds to isolate in the form of their 2,4-dinitrophenylhydrazones were furfural, diacetyl, and methylglyoxal (pyruvic aldehyde). Furfural DNPH occurs in two isomeric forms, of which the higher-melting form is poorly soluble in many solvents and difficult to elute from an adsorption column or to move on a paper chromatogram. Both diacetyl and methylglyoxal form bis-DNPH's, which are very difficultly soluble and which are held tenaciously on adsorption columns. These three derivatives were resolved successfully by adsorption on neutral alumina from a chloroform solution (necessarily dilute); the high-melting form of furfural DNPH was then displaced with 5% methanol in chloroform and the two bis-DNPH's with 10% pyridine in chloroform. Methylglyoxal bis-DNPH was the last to emerge, and only the 2,4-dinitrophenylhydrazine reagent itself is more strongly held by the adsorbent.

Furfural DNPH (high-melting isomer). Dark red crystals from pyridine, m.p. 223°C. Mixed m.p. with authentic sample, 227°C. M.p. of authentic sample, 233°C.

Diacetyl bis-DNPH. Orange powder from chloroform and petroleum ether, m.p. 317°C. Mixed m.p. with authentic sample, 316°C. M.p. of authentic sample, 318°C.

Methylglyoxal bis-DNPH. Very small orange needles from pyridine, m.p. 305°–309°C. Mixed m.p. with authentic sample, 308°C. M.p. of authentic sample, 308°C.

Identification of Organic Acids. Paper chromatograms of the ethylamine salts of volatile organic acids revealed the presence of acetic

acid with only traces of propionic acid. Since there was no evidence of free pyruvic or levulinic acids being present, it has been assumed that these occurred in the volatiles as esters. Not readily explainable is the fact that no other esters could be detected in a portion of the filtrate after removal of the carbonyl compounds as DNPH's.

Identification of Alcohols. The distilled filtrate from the preparation of the DNPH's contained a considerable amount of ethanol, easily detected by its odor. Treatment with 3,5-dinitrobenzoyl chloride and chromatography by the procedure of Holley and Holley (4) indicated that ethanol was by far the chief alcoholic constitutent in the volatiles; no other alcohol could be detected with confidence. A portion of the filtrate was saturated with magnesium sulfate, extracted with dry ether, the extract dried over anhydrous sodium sulfate and applied to a 5-foot column in a gas chromatograph (Aerograph Model A-100). The column packing was Carbowax 400 on C-22 firebrick, and operation was at 110°C. with helium as the carrier gas, at a flow rate of 24 ml. per minute. One distinct peak was obtained having a retention time identical with ethanol under the same conditions; at maximum sensitivity there was a barely perceptible "shoulder" on the peak, whose estimated retention time corresponded to that of isopropanol.

Quantitative Results. Expressed on the basis of one loaf of bread, the following concentrations were estimated: Titratable volatile acids (as acetic acid), 41 mg.; ethanol, 2.33 g.; furfural, 1.7 mg.; total dicarbonyls, 0.30 millimole; diacetyl, 1.44 mg. (0.017 millimole); methylglyoxal (0.30–0.017) = 0.28 millimole or 20 mg.

"Flavor Supplementation" Experiments. In the case where volatiles from conventional white bread were added as part of the ingredients for the chemically leavened bread, there was no significant effect on the flavor of the latter when compared with an unsupplemented control. The same was true when slices of the chemically leavened bread were treated with the vapors from conventional white bread.

Although the scanty quantitative data above are insufficient to permit formulation of a "synthetic flavor" composition, several such mixtures were made up arbitrarily, and the slice-aeration technique was used to assess their effects, if any, on flavor. The first mixtures tried comprised the compounds whose concentrations are listed above, together with various proportions of acetaldehyde, crotonaldehyde, 2-ethylhexanal, and hexanone-2. None of the mixtures was in the least breadlike in aroma. Substitution of pyruvic acid for acetic acid seemed to improve the odor, and the following mixture was used to aerate 12 slices of "instant" bread:

	ml
Ethanol (95%)	100.0
Furfural	0.37
Pyruvic acid	2.0
Diacetyl	0.27
Methylglyoxal (30% aq.)	0.72
2-Ethylhexanal	0.1
Crotonaldehyde	0.1

There was general objection to the overly prominent diacetyl odor of these slices. In view of the high volatility of diacetyl, this compound probably distilled into the slices in a disproportionate concentration. Two more mixtures were made up and tried, with this basic formulation:

	ml
Ethanol	100.0
Furfural	0.5
Pyruvic acid	2.0
Methylglyoxal (30% aq.)	0.8
Acetaldehyde	0.1
2-Ethylhexanal	0.2
Crotonaldehyde	0.2

This mixture was divided into two equal portions. To one was added 0.015 ml. diacetyl; to the second, 0.025 ml. Slices of "instant" bread were aerated with the two mixtures, and the flavors were compared with untreated slices by experienced personnel of the American Institute of Baking. No preferences were shown for any of the samples, although the aerated slices could be distinguished easily from the controls.

From the results of these experiments, it must be concluded that flavor fortification of chemically leavened bread by the means used is not practicable, at least with our present knowledge of bread flavor. It may be that some of the subtle characteristics of bread flavor are due to minute amounts of unidentified volatile substances. Furthermore, the natural states of flavorants in conventional bread may have much to do with the observed aroma and taste; it is extremely unlikely that compounds crudely sorbed onto bread slices will have the same relative vapor pressures as they would when produced naturally during fermentation and baking.

Summary

Several volatile compounds have been recovered and identified from fresh white bread, and some of these have been estimated. Neither the actual distillate containing these compounds nor several synthetic blends of them have proved of any value in enhancing the

palatability of a bland, chemically leavened bread. One conclusion seems inescapable; that certain products of fermentation, not necessarily the same as those identified here, are essential at the time a bread dough goes into the oven and are altered by oven heat (both in nature and amounts) to give rise to the actual constituents of flavor.

Literature Cited

1. Baker, J. C., and Mize, M. D. Some observations regarding the flavor of bread. Cereal Chem. **16:** 295–297 (1939).
2. Baker, J. C., Parker, H. K., and Fortmann, K. L. Flavor of bread. Cereal Chem. **30:** 22–30 (1953).
3. Hiscox, E. R., and Berridge, N. J. Use of the paper partition chromatography in the identification of the volatile fatty acids. Nature **166:** 522 (1950).
4. Holley, A. D., and Holley, R. W. Identification of alcohols in dilute aqueous solution. Anal. Chem. **24:** 216–218 (1952).
5. Horner, L., and Kirmse, W. Oxidative dealkylation of mixed substituted tertiary amines with dibenzyl peroxide. Annalen **597:** 48–65 (1955).
6. Huelin, F. E. Volatile products of apples. III. Identification of aldehydes and ketones. Australian J. Sci. Research **B5:** 328–334 (1952).
7. Huntress, E. H., and Mulliken, R. S. Identification of pure organic compounds. Wiley: New York (1941).
8. Jackson, E. L. Periodic acid oxidation. *In* Organic Reactions, vol. II, chap. 8, pp. 341–375. Wiley: New York (1944).
9. Johnson, G. D. A new preparation of 2,4-dinitrophenylhydrazones. J. Am. Chem. Soc. **73:** 5888–5889 (1951).
10. Johnson, G. D. Correlation of color and constitution. I. 2,4-dinitrophenylhydrazones. J. Am. Chem. Soc. **75:** 2720–2723 (1953).
11. Jones, L. A., Holmes, J. C., and Seligman, R. B. Spectrophotometric studies of some 2,4-dinitrophenylhydrazones. Anal. Chem. **28:** 191–198 (1956).
12. Kretovich, V. L., Tokareva, R. R., Petrova, I. S., and Drozdova, T. V. Aromatic substances of red rye malt and rye bread. Referat. Zhur. Khim. **1954** No. 39804.
13. Malmberg, E. W. A chromatographic study of carbonyl compounds present in a hydrocarbon-air flame. J. Am. Chem. Soc. **76:** 980–983 (1954).
14. Matz, S. A., Miller, J., and Davis, J. Modern baking concepts for troop feeding: Development of the instant bread mix. Food Technol. **12:** 625–629 (1958).
15. Rotsch, A. Über die Natur der beim Backen entstehenden Aromastoffe. [Aroma compounds produced during baking.] Brot u. Gebäck **10:** 162–166 (1956).
16. Stadtman, F. H. The chemical deterioration of dried fruits during storage. III. Chromatographic separation of carbonyl compounds as 2,4-dinitrophenylhydrazones. J. Am. Chem. Soc. **70:** 3583–3586 (1948).
17. Steinberg, M. A., Livingston, G. E., and Fellers, C. R. Chemical aspects of pectin gel darkening. Food Technol. **10:** 470–476 (1956).
18. Strain, H. H. 3-Nitrobenzohydrazones, 2,4-dinitrophenylhydrazones and the separation of hydrazones by adsorption. J. Am. Chem. Soc. **57:** 758–761 (1935).
19. Thomas, B., and Rothe, M. Formation of aldehydic aromatic substances during baking of bread. Ernährungsforschung **2:** 427–443 (1957).
20. Visser't Hooft, F., and deLeeuw, F. J. G. The occurrence of acetylmethylcarbinol in bread and its relation to bread flavor. Cereal Chem. **12:** 213–229 (1935).
21. Westerfeld, W. W. A colorimetric determination of blood acetoin. J. Biol. Chem. **161:** 495–502 (1945).

INTERCOMPARISON OF FARINOGRAPH ABSORPTION OBTAINED WITH DIFFERENT INSTRUMENTS AND BOWLS[1]

I. HLYNKA

ABSTRACT

A method for precise intercomparison of different farinographs is described. The technique is illustrated by data on combinations of two farinograph instruments and three mixing bowls. Linear regression equations for the mobility-absorption relation were established, using the same flour. From these data precise values for absorption were evaluated corresponding to the 500 B.u. consistency. Conversely, values of consistency corresponding to a selected value of absorption were also evaluated.

One of the major uses of the farinograph in cereal laboratories is for the determination of flour absorption (1). Operationally defined, flour absorption is the amount of water required to obtain a farinograph curve that is centered about the 500 unit line at maximum consistency. With a given instrument this determination can be performed with adequate precision. However, the divergence of data obtained with different instruments or bowls points to the need for standardization of farinographs[2] and for methods of precise comparison of different instruments and bowls.

At the present time it may be considered that two methods are available for the standardization and intercomparison of farinographs. In the first, replicate data can be obtained with the same flour for each bowl or instrument, and a correction factor can be established. In the second, mechanical adjustments may be made by the manufacturers (and other knowledgeable persons) to reduce the differences between bowls. An alternate method to the first is suggested by the work recently published by the author (2). This method makes use of the linear relationship that has been established between absorption and dough mobility. Linear regression equations can be established for each instrument and bowl, and precise correction factors can then be evaluated from these equations. To illustrate the method, this paper presents data obtained with two different instruments and three different bowls.

Materials and Methods

Two different farinographs were used. Farinograph I was a new,

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[2] The AACC has a Farinograph Standardization Committee.

variable-speed machine set to operate at 62.9 r.p.m. to match another new constant-speed machine available in this laboratory. Farinograph II was an old, prewar machine manufactured in Germany. Its speed was 59.3 r.p.m.

Three farinograph mixing bowls used with each of the two machines were: a small stainless-steel-clad bowl, A; a small solid stainless bowl, B; and a large nickel-plated bronze bowl, C.

The flour used in this study was an unbleached, improver-free, straight grade sample commercially milled from a blend of Canadian hard red spring wheat. The protein content was 12.2% and ash 0.47% on a 14% moisture basis.

The constant flour weight procedure (1) was used to obtain farinograph curves at varying absorption to give a range of dough consistencies from approximately 400 to 600 Brabender units. The same flour was used for all tests.

The values of dough consistency were taken as the ordinate about which the curve on the farinograph chart was centered at maximum consistency and were read to the nearest 5 Brabender units. The data were plotted as dough mobility (2), obtained as reciprocal of consistency, against percent absorption on a 14% moisture basis.

Results

The aim of the experiments was to obtain comparable absorption-mobility data with the same flour for two different farinographs and three different mixing bowls. The data were then used to obtain precise data for intercomparison of different instrument and mixing bowl combinations.

The data for farinograph I are summarized graphically in the upper half of Fig. 1. The two small bowls, A and B, gave results that were fairly close together, while the large bowl, C, gave quite different results.

Analogous data for the same three bowls but with farinograph II, which had a slower speed, are shown in the lower half of Fig. 1. All the curves are displaced downward in comparison with the corresponding curves obtained with farinograph I, and the difference between the large bowl and the two small bowls is increased.

Table I summarizes the regression equations for each instrument and bowl combination. The precision of the data is given by the standard deviation from the regression line or the standard error of estimate shown in the last column. The precision of the data for the small bowl is very high. It may be recalled, for example, that 0.06% difference in absorption (top entry) represents only 0.03 ml. in terms

TABLE I

SUMMARY OF ABSORPTION-MOBILITY DATA FOR DIFFERENT FARINOGRAPHS AND BOWLS

INSTRUMENT AND BOWL		REGRESSION EQUATION (Absorption = m Mobility + b)	STANDARD ERROR OF ESTIMATE (As % Absorption)
Farinograph I			
Small stainless-steel-clad bowl	(A)	$y = 8{,}334\,x + 43.69$	0.06
Small solid stainless-steel bowl	(B)	$y = 8{,}353\,x + 43.23$	0.07
Large nickel-plated bronze bowl	(C)	$y = 8{,}425\,x + 44.99$	0.14
Farinograph II			
Small stainless-steel-clad bowl	(A)	$y = 7{,}399\,x + 43.58$	0.09
Small solid stainless-steel bowl	(B)	$y = 7{,}185\,x + 43.50$	0.11
Large nickel-plated bronze bowl	(C)	$y = 8{,}025\,x + 45.11$	0.13

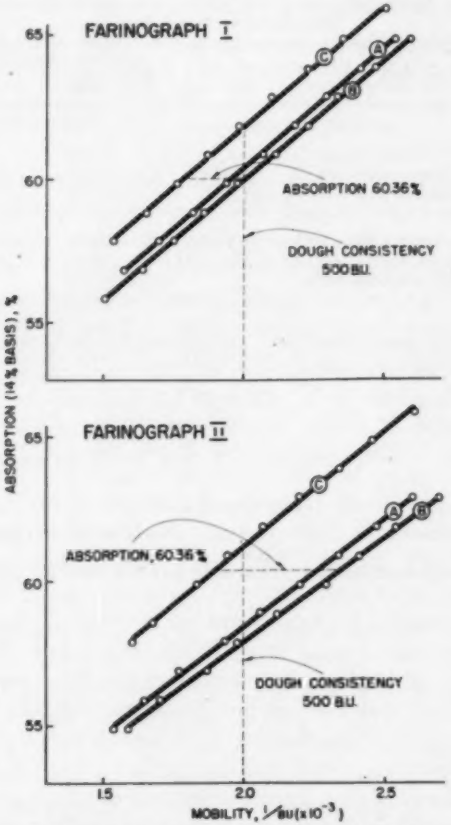

Fig. 1. Graphs of absorption-mobility relations obtained with the same flour for two different farinographs and three bowls.

of water added to 50 g. flour. On the other hand an error of 0.14% absorption for the large bowl (third entry) represents 0.42 ml. in terms of water added to 300 g. flour.

A comparison of any instrument-bowl combination may be made in two ways. The absorptions may be evaluated corresponding to a consistency of 500 B.u., shown in Fig. 1 by the vertical dashed line. Alternately, a fixed absorption (shown by the horizontal dashed line) may be selected, and the corresponding consistencies may be evaluated from the appropriate regression equations. These comparisons are summarized in Table II. The data are self-explanatory.

TABLE II

SUMMARY OF ABSORPTION AND CONSISTENCY DATA FOR INTERCOMPARISON OF FARINOGRAPHS AND BOWLS

INSTRUMENT AND BOWL		ABSORPTION (14% Basis) CALCULATED AT 500 B.U.	CONSISTENCY CALCULATED AT 60.36% ABSORPTION
		%	*B.u.*
Farinograph I			
Small stainless-steel-clad bowl	(A)	60.36	500
Small solid stainless-steel bowl	(B)	59.94	488
Large nickel-plated bronze bowl	(C)	61.84	548
Farinograph II			
Small stainless-steel-clad bowl	(A)	58.38	441
Small solid stainless-steel bowl	(B)	57.87	426
Large nickel-plated bronze bowl	(C)	61.16	526

The method just described should make it possible to obtain data for a precise intercomparison of different farinographs or bowls in the same laboratory, or in different laboratories, provided that the same flour is used. Moreover, this method should assist in the eventual standardization of farinographs to a more uniform basis.

Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. HLYNKA, I. Dough mobility and absorption. Cereal Chem. **36**: 378–385 (1959).

THE EFFECT OF THE DEGREE OF POLISHING OF RICE ON NITROGEN AND MINERAL METABOLISM IN HUMAN SUBJECTS[1]

G. RAMA RAO, H. S. R. DESIKACHAR, AND V. SUBRAHMANYAN

ABSTRACT

Negative calcium balances were recorded in human subjects when brown (unpolished) rice or rice polished to remove 2.9% of the brown rice was fed as the cereal portion in a poor vegetarian diet. Under similar conditions, rice polished to remove 4.1 or 6.3% of the brown rice produced slight positive calcium balances. In spite of its higher protein and phosphorus content, the brown rice did not produce higher nitrogen or phosphorus balances than the polished rice samples.

Rice polished to remove 4.1% of the brown rice represented a *via media* stage of polishing with 1.7 γ per g. of thiamine and could be recommended for consumption by rice eaters. The exclusive consumption of brown rice in diets containing marginal or submarginal amounts of calcium is not to be recommended as it may produce negative calcium balances.

It is well known that the polishing of rice leads to extensive loss of minerals and B-group vitamins and, hence, intake of undermilled rice is recommended on nutritional grounds. For reasons of economy also, completely unpolished rice is often given in the diet. Against these apparent advantages, brown rice or undermilled rice has poor storage and cooking qualities and contains large amounts of phytic acid which interferes with the utilization of dietary calcium. The latter aspect is all the more disturbing, since rice contains far less calcium than wheat (12) and is the staple diet for a large section of the people in many countries. People of the low-income groups in these countries consume very little milk and derive very little of their calcium requirements from any other source. This is particularly true of the "poor South-Indian rice diet" of India (2).

In rat experiments, brown rice had a higher growth-promoting capacity than polished rice (17). The biological value of the protein was also relatively higher for the brown rice (11). Human metabolism experiments, however, have shown that brown rice produces lower nitrogen and mineral balances than polished rice (4,5). This difference in response of the human being and the rat and the fact that rice forms over 75% of the diet of the low-income groups in India led to a reinvestigation of the problem by this laboratory, with a view to arriving at an optimal degree of polishing for rice which would not

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deplete thiamine below a safe level (about 1.5–1.8 γ per g.) and would not affect adversely mineral and nitrogen balances in human subjects. The problem is analogous to the effect of the degree of extraction of wheat flour on the nutritive value of bread, which has been investigated in great detail (19).

Metabolism studies on children which formed a part of these investigations on rice were completed first and have already been reported (10). Results of studies on adults using samples of rice milled to known degrees of polishing are reported here.

Materials and Methods

Healthy human adults from the laboratory served as experimental subjects for the study. The details of height and weight are given in Table I.

A long, fine-grained variety of rice (Bangar Sanna Co-11), shelled in a commercial sheller, was polished in a cone polisher to remove 2.9, 4.1, and 6.3% of the brown rice. The chemical composition of samples is given in Table II. These samples, hereafter referred to as 2.9-, 4.1-,

TABLE I

AGE, HEIGHT, AND WEIGHT OF THE EXPERIMENTAL SUBJECTS

SUBJECT NO.	AGE	HEIGHT	WEIGHT
	years	*meters*	*kg*
1	30	1.62	45.4
2	30	1.60	50.9
3	26	1.62	50.0
4	32	1.68	49.1
5	28	1.60	52.7
6	26	1.54	45.4
7	24	1.58	55.0

TABLE II

CHEMICAL COMPOSITION AND OTHER CHARACTERISTICS OF RICE POLISHED TO DIFFERENT DEGREES

	6.3%-POLISHED RICE	4.1%-POLISHED RICE	2.9%-POLISHED RICE	BROWN RICE
Chemical composition				
Nitrogen, mg%	936.0	963.0	987.0	1,014.0
Calcium, mg%	8.9	9.3	11.1	12.4
Total phosphorus, mg%	215.0	245.0	296.0	365.0
Phytin phosphorus (as % of total phosphorus) mg%	38.6	52.2	56.1	60.3
Crude fiber, %	0.20	0.22	0.50	0.80
Thiamine, γ/g	1.1	1.7	2.1	3.2
Cooking quality	Good	Good	Reasonable	[a]
Storage quality	Good	← Intermediate →		[b]

[a] Unacceptable. A stiff peel or coat of bran is formed.
[b] Prone to insect damage; develops rancidity.

and 6.3%-polished rice, together with shelled rice from the same variety, formed the cereal portion in the diet fed to the experimental subjects.

The diet was similar in other respects to the "poor South-Indian diet" (3) which has been adopted as typical of what is consumed by the majority of rice eaters belonging to the low-income groups in South India (2). It is mainly vegetarian and contains very little milk, and for this reason is very deficient in calcium. Ingredients of the daily diet per individual were:

	g.
Rice	500.0
Thur dhal (*Cajanus cajan*)	50.0
Ground nut oil	50.0
Leafy vegetable (*Amaranthus tricolor*)	21.0
Nonleafy vegetables (potato and brinjal)	82.0
Whole milk powder (Nespray)	9.0
Cane sugar	35.0
Other pulses (Bengal gram, black gram, etc.)	6.6
Flavoring and spicing ingredients, including coffee beverage	26.5
Common salt	20.6
(Total calories, 2,900 to 3,000)	

Chemical composition: protein (N × 6.25), 48.7–51.2 g.; fat, 55–62 g.; calcium, 401–419 mg.; phosphorus, 1,431–2,183 mg. (The change in the degree of polishing of rice used in the feeding periods causes slight variation.)

The daily feeding pattern consisted of breakfast, lunch, tea, and dinner. A rice semolina or flour preparation along with coffee was served at breakfast; cooked rice, spiced vegetable gravy (*sambar*), and buttermilk were given at lunch and dinner. Coffee was served between lunch and dinner.

The general plan of the experiments was to feed the above rice diet for 12 days to the subjects and collect excretions in the last 5 days of the feeding period for analysis. The dietary regimen during the four consecutive feeding periods was identical except for a change in the degree of polishing of the rice forming the main constituent of the diet. A week's rest period was allowed for the subjects between two metabolism periods. In order to get the subjects slowly adapted from the polished to the undermilled or brown rice in the diet, the 6.3%-polished rice was fed in the first experimental period. The 4.1%- and 2.9%-polished rices and brown rice were fed in the second, third, and last periods, respectively. The same subjects were used for the four metabolism periods, although one of the subjects dropped out of the experiment for the last two periods for unavoidable reasons.

The 24-hour urine and fecal samples for each subject collected and preserved under antiseptic conditions (acid was also used to bind free ammoniacal nitrogen) were pooled together for 5 days for subsequent analysis. Carmine was used as feces-marker. Aliquots of urine and dried fecal samples were used for analysis of nitrogen, calcium, and phosphorus. Identical portions of diet servings fed at each sitting were removed and dried. The dried total diet for each experimental period was mixed and pulverized, and aliquots were used for analysis.

In representative samples of the rice, diet, and excretions, the following were determined:

1) Nitrogen (by micro-Kjeldahl procedure)
2) Calcium; experimental materials subjected to dry ashing, taken up with hydrochloric acid and determined by oxalate precipitation (1)
3) Phosphorus; materials solubilized by wet digestion according to Gerritz (7) and estimated by the colorimetric procedure of Fiske and Subbarow (6)
4) Phytin phosphorus, by the method of McCance and Widdowson (13)
5) Thiamine by the thiochrome method as modified by Kik and Williams (12)

Results and Discussion

From the point of view of chemical composition, there is a gradual loss of all nutrients with progressive polishing of the rice (Table II), the maximum losses being found in thiamine (65%) and phosporus (40%). Loss of protein content due to polishing is, however, less than 10% even at the highest level of polishing. The total phosphorus, as well as the phytin phosphorus, decreases during the polishing process.

The diet used in these experiments contained other minor constituents, but rice formed over 70% of the total diet; therefore the effects observed may be ascribed largely to the rice in the diet, and the differences in the results obtained with the different diets may be ascribed to changes in the degree of polishing of the rice. Results (Table III) show the effect of polishing on the balances of nitrogen, calcium, and phosporus. In spite of slightly higher intakes of nitrogen in the diet as a result of their higher protein content, the brown and undermilled samples of rice did not produce greater nitrogen balances than the better-polished samples. Large individual variations among subjects in nutrient balances (see Table III) have precluded a strict statistical analysis of the data; even so, the results indicated a slightly higher nitrogen balance in the polished rice diet (Cullumbine

et al., 4,5). In any case, the polished rice did not produce a lower nitrogen balance than the brown rice or the two undermilled rice samples. The results of calcium balances are more striking. Brown rice and 2.9%-polished rice produced a slightly negative average calcium balance (four subjects on each of the diets had negative balances), whereas 4.1%-polished and 6.3%-polished rice induced small positive balances of the order of 36 and 77 mg., respectively. Positive phosphorus balances were recorded for all the subjects during each of the four dietary periods, although the underpolished samples did not produce higher balances as would be expected merely on the basis of their much higher total phosphorus contents. In general, these results are similar to those obtained by Kantha Joseph *et al.* (10) on the effect of feeding children rice polished to different degrees.

These results on calcium and phosphorus balances are explained on the basis of the higher content of phytin phosphorus in the samples with a low degree of polishing. Although a fair amount of phytin phosphorus is hydrolyzed in the intestines (5,14,16) as a result of gastrointestinal enzymes or enzymes of bacterial origin in the lower part of the intestines, it exerts a deleterious effect on calcium absorption. The higher fibrous and indigestible residue in unpolished rice is also responsible for the apparent low digestibility of the nutrients on such

TABLE III

AVERAGE DAILY INTAKE AND BALANCE OF NUTRIENTS ON DIETS BASED ON RICE
(Variability is expressed in terms of standard error of the mean values)

DEGREE OF RICE POLISH AND NO. OF SUBJECTS[a]	INTAKE	EXCRETION			BALANCE
		Urinary	Fecal	Total	
			Nitrogen, g.		
Brown — 6	8.19	4.32 ± 0.21	3.25 ± 0.15	7.57	0.62 ± 0.31
2.9% — 6	7.95	4.36 ± 0.23	2.74 ± 0.15	7.10	0.85 ± 0.04
4.1% — 7	7.93	4.43 ± 0.21	2.73 ± 0.19	7.16	0.77 ± 0.08
6.3% — 7	7.80	4.18 ± 0.29	2.76 ± 0.07	6.94	0.86 ± 0.24
			Calcium, mg.		
Brown — 6	419	63 ± 8.8	361 ± 11.1	424	−5 ± 6.9
2.9% — 6	411	60 ± 8.9	357 ± 32.9	417	−6 ± 27.9
4.1% — 7	403	73 ± 10.9	294 ± 16.4	367	36 ± 13.7
6.3% — 7	401	42 ± 8.7	282 ± 19.5	324	77 ± 15.5
			Phosphorus, mg.		
Brown — 6	2,183	479 ± 24.8	1,362 ± 15.3	1,841	342 ± 29.5
2.9% — 6	1,804	429 ± 23.8	955 ± 96.1	1,384	420 ± 63.7
4.1% — 7	1,581	422 ± 22.4	785 ± 18.3	1,207	374 ± 23.0
6.3% — 7	1,431	408 ± 18.5	588 ± 25.3	996	435 ± 26.3

[a] The successive order of feeding of the rice samples was, as stated in the text, in decreasing order of the degree of polishing of the rice. The data are, however, presented in reverse order for convenience.

TABLE IV
APPARENT DIGESTIBILITY OF NUTRIENTS

TYPE OF RICE USED IN DIET	DRY FECAL BULK	APPARENT DIGESTIBILITY		
		Nitrogen	Calcium	Phosporus
	g/day	%	%	%
Brown	64.1	60.3	13.9	37.6
2.9%-Polished	50.4	64.5	12.8	48.5
4.1%-Polished	50.7	65.6	27.1	50.3
6.3%-Polished	46.3	64.6	28.2	58.9

diets (see Table IV). The fecal bulk on the dry basis was much higher on the unpolished rice diet. Differences between 6.3-, 4.1-, and 2.9%-polished rice were, however, small, indicating that the type of fiber which increases fecal bulk is removed much faster in the earlier stages of the polishing itself.

Brown rice and 2.9%-polished rice have poor culinary and storage qualities and also produced negative calcium balances when fed in the rice diet. The 6.3%-polished rice is otherwise acceptable in every respect, but its thiamine content is the lowest (1.1 γ per g.). Even so, it can reasonably be expected to retain a large amount of this thiamine in the cooked state, provided washing losses are eliminated. The 4.1%-polished rice can be considered a *via media* stage of polishing. Although it has a dull appearance and is not suitable for prolonged storage, it is, on the whole, the best from the nutritional point of view. For those sections of the people whose diet contains other sources of thiamine, the fully polished rice may be adequate; while for those whose intake of thiamine is dependent largely on the rice in the diet, a 4%-degree of polishing can be recommended (slightly higher extraction rate may be allowed for coarse varieties with thicker bran layers). In spite of its high thiamine content, the use of unpolished rice or rice with a very low degree of polishing (below 2–3%) cannot be recommended unreservedly in view of its deleterious effect on dietary calcium absorption. The consumption of "parboiled," "converted," or "malekized" rice which retains higher proportions of thiamine, even when polished, is a safer and more suitable means of meeting the thiamine requirements than the exclusive intake of brown rice.

The capacity of human subjects to maintain small positive balance even at low levels of calcium intake and their ability to adapt themselves to low levels of dietary calcium has been demonstrated in Ceylonese (15), African (18), and Peruvian adults (9). This has more recently been demonstrated in dogs also (8). In the present experiments, as well as in similar experiments on children (10), negative

calcium balances have been found in many of the subjects fed brown or 2.9%-polished rice. For this reason, the feeding of completely unpolished rice over prolonged periods, especially to children in the growing age group, may lead to slow depletion of body calcium and should be viewed with caution. In such borderline cases, at least, the possible need for supplementing the diet with extra calcium and the best means of doing it should be kept in mind.

Literature Cited

1. Association of Official Agricultural Chemists. Official methods of analysis of the AOAC (8th ed.). The Association: Washington, D. C. (1956).
2. Aykroyd, W. R., Krishnan, B. G., Passmore, R., and Sundararajan, A. R. The rice problem in India. Indian Med. Research Mem. No. 32 (1940).
3. Council of Scientific and Industrial Research (India). Investigation on the composition and nutritive value of vanaspati. New Delhi, India (1952).
4. Cullumbine, H. Nitrogen balance studies on rice diets. Brit. J. Nutrition **4:** 129–133 (1950).
5. Cullumbine, H., Basnayake, V., Lemottee, J., and Wickramanayake, T. W. Mineral metabolism on rice diets. Brit. J. Nutrition **4:** 101–111 (1950).
6. Fiske, C. H., and Subbarow, Y. The colorimetric determination of phosphorus. J. Biol. Chem. **66:** 375–400 (1925).
7. Gerritz, H. W. A rapid method of preparing biological materials for phosphorus determination. Ind. Eng. Chem., Anal. Ed. **7:** 116–118 (1935).
8. Gershoff, S. N., Legg, M. A., and Hegsted, D. M. Adaptation to different calcium intakes in dogs. J. Nutrition **64:** 303–312 (1958).
9. Hegsted, D. M., Moscoso, Irma, and Collazos, C. A study of the minimum calcium requirements of adult men. J. Nutrition **46:** 181–201 (1952).
10. Kantha Joseph, Narayana Rao, M., Sita Ganapathy, Swaminathan, M., and Subrahmanyan, V. Studies on the nutritive value of rice and rice diets. II. Metabolism of nitrogen, calcium, and phosphorus in children on poor vegetarian diets containing husked, undermilled, and milled raw rice. Ann. Biochem. and Exp. Med. (Calcutta) **18:** 51–58 (1958).
11. Kik, M. C. The biological value of the proteins of rice and its by-products. Cereal Chem. **16:** 441–447 (1939).
12. Kik, M. C., and Williams, R. R. The nutritional improvement of white rice. Bull. No. 112, National Research Council Committee on Cereals, Food and Nutrition Board. Washington, D. C. (1945).
13. McCance, R. A., and Widdowson, Elsie M. Phytin in human nutrition. Biochem. J. **29:** 2694–2699 (1935).
14. McCance, R. A., and Widdowson, Elsie M. Mineral metabolism of healthy adults on white and brown bread dietaries. J. Physiol. **101:** 44–85 (1942).
15. Nicholls, L., and Nimalasuriya, Ananda. Adaptation to a low calcium intake in reference to the calcium requirements of a tropical population. J. Nutrition **18:** 563–577 (1939).
16. Subrahmanyan, V., Narayana Rao, M., Rama Rao, G., and Swaminathan, M. The metabolism of nitrogen, calcium, and phosphorus in human adults on a poor vegetarian diet containing ragi (*Eleusine coracana*). Brit. J. Nutrition **9:** 350–357 (1955).
17. Subrahmanyan, V., Narayana Rao, M., and Swaminathan, M. Studies on the nutritive value or rice and rice diets. I. The nutritive value of husked, undermilled and milled raw rice. Ann. Biochem. and Exp. Med. (Calcutta) **16:** 81–88 (1956).
18. Walker, A. R. P., Fox, F. W., and Irving, J. T. Studies in human mineral metabolism. I. The effect of bread rich in phytate phosphorus on the metabolism

of certain mineral salts with special reference to calcium. Biochem. J. 42: 452–462 (1948).

19. WIDDOWSON, ELSIE M., and MCCANCE, R. A. Studies on the nutritive value of bread and on the effect of variations in the extraction rate of flour on the growth of undernourished children. [British] Medical Research Council. Special Report Series No. 287. H. M. Stationery Off. (London) (1954).

NOTE ON THE IRRADIATION OF FLOURS FROM THIRTEEN VARIETIES OF WHEAT[1]

C. C. LEE[2]

In a previous paper (2), it was reported that the irradiation of flour with Co^{60} gamma rays caused a decrease in the recovery of crude gluten and an increase in the maltose value, the latter attributable to enhanced susceptibility of the starch, after irradiation, to autolytic hydrolysis. In the present work, a study was made on possible varietal differences in the changes brought about by irradiation. Samples of flour from 13 varieties of wheat with widely different breadmaking quality were each irradiated with 700,000 r. of Co^{60} gamma rays. These varieties, together with arbitrary rankings of their baking quality are listed in Table I. The irradiation was carried out by placing the flour in covered 50-ml. beakers and setting these beakers in fixed positions near a 90-curie Co^{60} source. The dose rates were measured by determining the ferric ions formed upon irradiating solutions of ferrous ammonium sulfate (5). Under the conditions of the experiments, exposures of the order of 17 hours were required to give the 700,000-r. dosage.

Recoveries of crude gluten and maltose values were determined on all samples of flour before and after irradiation, as outlined in *Cereal Laboratory Methods* (1). The results are also given in Table I. As expected, flours from all 13 varieties, after irradiation, showed decreased gluten recoveries and increased maltose values. There are considerable similarities in the magnitudes of these changes, as shown in columns 5 and 8 of Table I. After irradiation with a dose of 700,000 r., the loss

[1] Manuscript received March 31, 1959.
[2] Department of Chemistry, University of Saskatchewan, Saskatoon, Sask.

in gluten recovery was within 2.4–3.0% for nine of the 13 varieties, while increases in maltose value were within the range of 31–40 mg. maltose per 10 g. flour for seven varieties and within the range of 46–52 mg. maltose per 10 g. flour for four others. Such similarities suggest that the gamma rays very probably caused random fragmentations of the protein and starch. The extent of breakdown of such molecules will be dependent on total radiation dosage and will not be very discriminating among flours of different breadmaking quality. Recently, Maes (4) reported that radiation had an improving effect on flours from certain weak European wheats, and hinted that radiation treatment has a special virtue for such wheats which it might not have for other types of stronger wheat. The present data, however, appear to indicate a similarity in the response to radiation for flours from widely varying types of wheat.

The breakdown of flour proteins by the gamma rays was also studied in another way. Samples of flour before and after irradiation were fractionated according to a procedure similar to that reported by McConnell and Ramachandran (3). Briefly, this consisted of extracting a 5-g. sample of flour first with a 5% solution of potassium sulfate. This extract was separated into two fractions by treatment with 10% trichloroacetic acid (TCA). The precipitated protein was designated "albumin" and the supernatant liquid termed "water-soluble nonprotein

TABLE I

GLUTEN RECOVERIES AND MALTOSE VALUES FOR FLOURS BEFORE AND AFTER IRRADIATION WITH 700,000 ROENTGENS OF Co^{60} GAMMA RAYS

RANK[a] AND VARIETY	GLUTEN RECOVERY[b]			MALTOSE VALUE[b]		
	Before Irradiation	After Irradiation	Difference	Before Irradiation	After Irradiation	Difference
	%	%	%	*mg/10g flour*	*mg/10g flour*	*mg/10g flour*
1 — Pacific Club	6.2	3.2	3.0	89	116	27
2 — Rio Negro	18.4	16.0	2.4	115	134	19
3 — McMurachy × Exchange	14.2	11.2	3.0	231	282	51
4 — Centana	12.3	9.7	2.6	161	213	52
5 — Kenya 321	11.7	9.3	2.4	188	235	47
6 — Redman	14.3	11.5	2.8	152	188	36
7 — Apex	13.3	11.9	1.4	161	207	46
8 — Mida	12.8	10.0	2.8	164	204	40
9 — Lake	12.8	10.3	2.5	198	234	36
10 — Rescue	13.0	10.5	2.5	170	201	31
11 — Rescue × Chinook	11.0	9.1	1.9	226	264	38
12 — Chinook	14.7	13.3	1.4	156	190	34
13 — Thatcher	12.7	10.9	1.8	149	188	39

[a] Arbitrary ranking of breadmaking quality for the 13 varieties, higher ranks being given for better quality.

[b] Mean values of two to four determinations.

nitrogen." The flour remaining after the extraction with the potassium sulfate solution was next extracted with 0.02*N* sodium hydroxide. This alkaline extract was also treated with 10% TCA. The precipitated protein was designated "gluten" and the aqueous residue called "alkali-soluble nonprotein nitrogen." The material remaining after the two extractions was termed the "residue." Kjeldahl nitrogen determinations were carried out on all the fractions as well as on the original flour. As an illustration, a typical set of results, that for the flour from the variety Rescue (rank No. 10), is given in Table II. For all 13 varieties,

TABLE II

CRUDE PROTEIN CONTENTS (N × 5.7) OF FRACTIONS FROM FLOUR OF RESCUE WHEAT

	Protein Content						
	Original Flour	Albumin	Water-Soluble Nonprotein Nitrogen (N × 5.7)	Gluten	Alkali-Soluble Nonprotein Nitrogen (N × 5.7)	Residue	Total Recovery
	%	%	%	%	%	%	%
Before irradiation	13.8	1.2	1.0	10.9	0.4	0.4	13.9
After irradiation	13.7	0.4	1.8	9.6	1.4	0.4	13.6

irradiation caused decreases in the albumin and gluten fractions and increases in the fractions containing the water-soluble and alkali-soluble nonprotein nitrogen, thus strongly indicating breakdown of the proteins by gamma rays.

Acknowledgments

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Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. LEE, C. C. The baking quality and maltose value of flour irradiated with Co^{60} gamma rays. Cereal Chem. **36**: 70–77 (1959).
3. MCCONNELL, W. B., and RAMACHANDRAN, L. K. Acetate metabolism of maturing wheat plants. Can. J. Biochem. Physiol. **34**: 180–190 (1956).
4. MAES, E. Influence des radiations ionisantes (rayons gamma) sur la valeur boulangère de la farine. Bull. ecole meunerie belge **20** (5): 60–65 (1958).
5. WEISS, J., ALLEN, A. O., and SCHWARZ, H. A. Use of the Fricke ferrous sulfate dosimeter for gamma-ray doses in the range 4 to 40 kr. Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1955, vol. 14. pp. 179–181 (1956).

THE PRODUCTION OF AMYLOSE AND AMYLOPECTIN IN CORN ENDOSPERMS AND IN POTATO TUBERS[1]

STIG R. ERLANDER[2]

ABSTRACT

Experimental evidence indicates that amylose and amylopectin are produced simultaneously. Consequently, any proposed mechanism for the synthesis of starch based on the assumption that the branching enzyme is inactivated at some time during the day, in order to enable the synthesis of amylose, would appear to be invalid. Amylose (3.1%) was produced in very immature waxy corn endosperm by covering the ears with cellophane bags. One can postulate that the production of starch by plants occurs via glycogen. That is, plant glycogen is attacked by a theoretical debranching enzyme which (a) removes the outer or available branches of the glycogen to form amylopectin and then (b) connects these removed branches end-to-end to form amylose. In normal waxy endosperm the absence of amylose can be explained by assuming the presence of an inhibitor of the proposed debranching enzyme. The production of amylose in very immature waxy corn endosperm indicates that the activity of this inhibitor may be diminished by retarding the growth of certain factors in the very immature waxy corn endosperm. The average chain length of amylose appears to increase with an increase in the average chain length of the corresponding amylopectin. These results can be explained by assuming that the degree of polymerization of the unit chains removed by the proposed debranching enzyme remains constant. Consequently, the degree of polymerization of an amylose appears to be a function of the chain length of its parent glycogen.

In most plants, starch-synthesizing cells produce both the linear and the branched components amylose and amylopectin. At present only two types of enzymes have been found to be directly connected with the synthesis of either starch or glycogen from glucose-1-phosphate: phosphorylase and the branching enzymes (2,10,16). The mechanisms of these enzymes are well known. Recently the role of phosphorylase in the synthesis of animal glycogen has been questioned (4). According to Niemeyer (see ref. 4), phosphorylase may only be used *in vivo* to degrade glycogen to glucose-1-phosphate instead of to aid in its synthesis. Glycogen is synthesized in *Neisseria perflava* by means of amylosucrase (11). Also, sucrose may be directly converted into starch, as suggested by Ewart *et al.* (8) and supported by Badenhuizen (1). It would appear, therefore, that the entire mechanism for the synthesis of starch is open to criticism. The common notion (2,10,12,16,18) that amylose is the precursor of amylopectin may be entirely false.

The most difficult problem in proposing a mechanism for the

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synthesis of starch is how to account for the presence of the linear component amylose in starch and its absence in animal glycogen. Whelan and Walker (20) have recently proposed a compartment method for the separate synthesis of amylose and amylopectin. The author (5) has proposed that plant glycogen is synthesized first and this glycogen is then converted into amylose and amylopectin by an unknown debranching enzyme.

To obtain information on the mechanism employed by plants to synthesize starch, experimental investigations were carried out along three lines: 1) the effect of light and dark on the total and relative amounts of amylose synthesized in the corn plant; 2) the effect of light on the synthesis of amylose in the potato starch; and 3) the effect of water evaporation from the kernel on the synthesis of amylose in the corn plant. Data are also included on the relationship between the degree of branching of the amylopectin and the molecular weight of the corresponding amylose in corn starches. The results of these investigations are discussed in the light of the author's (5) proposed mechanism for synthesis of starch from glycogen.

Experimental Work

Corn Varieties and Method of Sampling. The varieties of corn plants used, including their endosperm genealogy, were Seneca Chief sweet corn su_1, su_1, su_1, *Wx*, *Wx*, *Wx*; Iowa 4297 dent corn Su_1, Su_1, Su_1, *Wx*, *Wx*, *Wx*; and Iowax 5 hybrid waxy corn Su_1, Su_1, Su_1, *wx*, *wx*, *wx*. The sweet, dent, and waxy varieties were hand-pollinated, respectively, on July 20, 25, and 24 in 1955. All samples of a given variety were pollinated at the same time. Three samples were collected from each variety at 12-hour intervals on the 13th and 20th day after pollination: 13th morning, 13th evening, 14th morning, 20th morning, 20th evening, and 21st morning. A sample of each variety was also collected at maturity. The immature kernels were collected between 5:45 and 6:30 in the morning and between 5:45 and 6:30 in the evening. Each ear was first husked while on the plant, then picked, and then immediately shelled with a knife and frozen on solid carbon dioxide in order to keep enzyme degradation to a minimum. All immature samples were kept frozen on dry ice until they were processed for starch (no longer than 4 days after picking).

In order to study the effect of water evaporation from the kernel during plant growth, some samples that were picked on the 13th morning and 20th morning had been covered with cellophane bags and husked about the third or fourth day after pollination. Two cellophane bags, tied on by an elastic band, covered the ear. The outer bag

contained a small amount of water to retard evaporation. These samples were collected as above.

Isolation of Corn Starch Samples. The starch was isolated from the kernels in a room held at approximately 4°C. The frozen kernels were ground with a small amount of iced distilled water for 3 minutes in a Waring Blendor (16,21). The slurry was screened through No. 17 nylon bolting cloth. The magma was then squeezed as dry as possible. The press cake was ground for another 3 minutes with fresh water, then screened as before. The combined extracts were centrifuged in an International centrifuge. Microscopic examination of the separated granules indicated no detectable granule damage. The gluten in the starch was removed according to the method of Maywald, Christiansen, and Schoch (17). The above starch-gluten mixture was shaken with 20% Pentasol mixture for three or four 15-minute periods. Each period was followed by centrifuging, discarding the supernatant, and adding fresh 20% Pentasol solution. The final starch was washed with 95% ethanol and Soxhlet-extracted for 48 hours with 95% ethanol to remove the associated fatty acids. The starches were then air-dried and

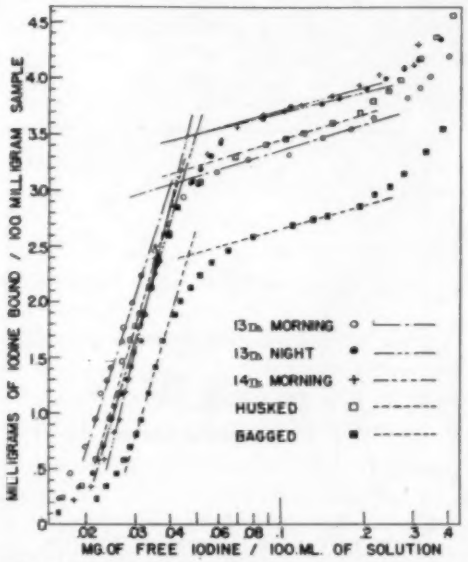

Fig. 1. Immature sweet corn starch samples. Iodine titration curves for the 13th morning, 13th night, 14th morning, husked ears, bagged ears (see legend and see text for the explanation of these starch samples). The intersection of the two lines for each titration curve gives mg. of iodine bound per 100 mg. of sample (iodine-binding capacity). The iodine titration curves for the other corn starch samples were similar to the above curves.

kept in closed containers. The mature samples were isolated in the same way without sulfur dioxide treatment, since it is believed that hydrolysis of the starch occurs with sulfur dioxide treatment.

Growth and Isolation of Potato Starch Samples. Certified Irish Cobbler potatoes were planted June 1, 1955, in three plots which had been equally fertilized and were located 3 to 5 feet apart. Four plants were planted in each plot. In the first plot the plants were exposed to natural sunlight throughout the day and to a fluorescent lamp at night. The adjustable light was held a few inches above the plants and was turned off for 2 hours each night. The second plot was exposed to the regular diurnal variations in light. The third plot was shaded so that the plants were exposed to sunlight for approximately 2 hours every afternoon.

The mature potato tubers were ground in a meat grinder. Water was added and the starch slurry was passed through No. 17 nylon bolting cloth. The same procedure as described above for separation of protein and fat was then followed.

Iodine Titration of Starch Samples. The percent amylose in all of the samples was determined according to the method of Lansky, Kooi, and Schoch (15), which is a modification of the method of Bates, French, and Rundle (3). The data, however, were graphed in a different manner as suggested by Dexter French[3] in order to increase the

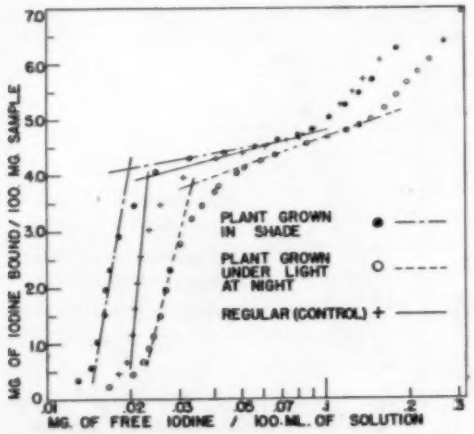

Fig. 2. Potato starch samples. Iodine titration curves for samples of mature potato starch produced under various conditions. The potato tuber starch samples were obtained from potato plants grown in the shade, grown under a fluorescent light at night and sunlight during the day, and grown under normal conditions as a control.

[3] D. French; private communication.

precision. A plot was made of mg. of iodine bound per 100 mg. sample versus the logarithm of mg. of free iodine per 100 ml. of solution. The intercept of the two lines gives the mg. of iodine bound per 100 mg. of sample. Typical plots for the corn and potato starch samples are given in Figs. 1 and 2. All of the samples were corrected for moisture content by drying representative samples in a vacuum oven at approximately 60°C. until a constant weight had been obtained (4 or 5 days).

Ferricyanide Number Determination. The sweet corn amylose was separated from the amylopectin by the method of Lansky, Kooi, and Schoch (15) after refluxing under a helium atmosphere in a buffered *n*-amyl alcohol solution for 24 hours (7). The adhering phosphate was removed by dialysis. The ferricyanide number was determined on the amylose solution according to the method described by Kerr (13). To determine the concentration, a weighed portion of the amylose solution was predried under a heat lamp and finally in a vacuum oven at 95°C. until constant weight was obtained.

Calculations

Let us assume that amylose is produced only during the daytime and amylopectin only during the night. Then from the percentages of amylose and the 24-hour yields given in Table I, we can calculate the percentage of amylose for the 13th night according to the equation:

$$A_{13N} = \frac{(Y_{14M} - Y_{13M})A_{14M} + Y_{13M}A_{13M}}{Y_{13M} + (Y_{14M} - Y_{13M})A_{14M}} \quad (1),$$

where

A_{13N} = predicted fraction of amylose for the 13th night, assuming that amylose is produced from 6 a.m. to 6 p.m and amylopectin from 6 p.m to 6 a.m.;

A_{13M} = fraction of amylose in the 13th morning sample;

A_{14M} = fraction of amylose in the 14th morning sample;

Y_{13M} = yield of starch in g. per ear for the 13th morning sample; and

Y_{14M} = yield of starch in g. per ear for the 14th morning sample.

Yields were expressed as g. per ear since this gives roughly the amount of starch per plant (or per kernel). To reduce the error in the yields, the 13th night yield was not used in the calculation. It was assumed in the above calculation that the increase in the percentage of amylose from the 13th morning sample to the 14th morning sample is negligible. From Table I we see that this assumption is valid.

Similar calculations were made for the 20th night samples by substituting the numbers 20 and 21 for 13 and 14, respectively, into equa-

tion 1, since these numbers designate the number of days after pollination.

Because of the possibility of a large error in the daily yields, calculations were also made from weekly yields. Various rates of starch production were assumed, since the exact rate of increase in starch per day for an average ear or kernel was not known. The rate constants were obtained by averaging in each case the three rate constants obtained from the 7-day increases: 13th morning to 20th morning, 13th night to 20th night, and 14th morning to 21st morning. The daily increases in starch yield ($Y_{14M} - Y_{13M}$ and $Y_{21M} - Y_{20M}$) were obtained from the calculated rate constants using the experimental values for Y_{13M} and Y_{20M}. In all of these calculations the experimental values for A_{13M} and A_{20M} were used, but the values for A_{14M} and A_{21M} were obtained from the observed weekly increase in percent amylose, assuming zero-order kinetics. Only a small error is involved in assuming a zero-

TABLE I

TWELVE-HOUR-PERIOD YIELDS FOR DENT, SWEET, AND WAXY CORN STARCH, AND THEIR IODINE-BINDING CAPACITIES

SAMPLE[a]	No. OF EARS	TOTAL STARCH YIELD[b]	PERCENT STARCH PRODUCED DURING DAYTIME[c]	IODINE BOUND PER 100 MG. OF SAMPLE	PERCENTAGE OF AMYLOSE[d]
		g/ear		*mg*	
Dent corn					
13th morning	10	6.67	...	3.40	17.90
13th night	10	6.27	−19.6	3.40	17.90
14th morning	10	8.31	...	3.55	18.70
20th morning	3	21.0	...	4.25	22.35
20th night	3	21.9	17.6	4.40	23.15
21st morning	3	26.1	...	4.48	23.60
Sweet corn					
13th morning	11	1.16	...	3.05	16.05
13th night	11	1.33	29.8	3.50	18.40
14th morning	11	1.73	...	3.48	18.30
20th morning	3	5.76	...	4.38	23.05
20th night	3	5.98	5.59	4.28	22.50
21st morning	4	9.70	...	4.35	22.90
Waxy corn					
13th morning	8	5.27	...	...	....
13th night	8	8.6	34.2	...	....
14th morning	8	15.0	...	...	....
20th morning	2	26.7	...	...	....
20th night	2	30.8	36.3	...	....
21st morning	2	38.0	...	...	....

[a] Numbers designate the number of days after pollination.

[b] After defatting and correcting for moisture content. The daily or 24-hour increases in starch yield were calculated by using only the morning samples (see text).

[c] These values were obtained directly from all of the experimental morning and night samples, for example, $100(1.33 - 1.16)/1.73 = 29.8\%$ for the 13th-night sweet corn starch yield. The values were not used to calculate the amount of amylose produced during the daytime because of the possibility of a large error, i.e., the difference between the 13th or 20th morning starch sample yields and their corresponding night samples is small (see text).

[d] Assuming that pure amylose binds 19 mg. of iodine per 100 mg. of sample.

order increase in amylose because of the small weekly increase in percent amylose during this period.

The percentage of amylose in all samples was calculated, assuming that pure amylose has an iodine-binding capacity of 19 mg. of iodine per 100 mg. of sample. This assumes that any change in the molecular weight of the amylose does not change the iodine-binding capacity (13). It was also assumed that any protein impurities present in the starch bound iodine to the same extent in all of the starches.

Results and Discussion

Production of Starch in Corn Endosperms. A comparison of the amount of experimentally determined amylose with the amount predicted, assuming that amylose is produced during the daytime and amylopectin during the night, is made in Table II. The theoretical values were calculated as discussed above. The precision of the iodine affinity determination according to Lansky *et al.* (15) should be $\pm 0.08\%$. The estimated error in the theoretical values in Table II is approximately 10% in all cases. Therefore the differences in the predicted and experimental values are real.

TABLE II

COMPARISON OF EXPERIMENTAL AND PREDICTED PERCENT AMYLOSE, ASSUMING DAYTIME PRODUCTION OF AMYLOSE

CORN STARCH SAMPLES	EXPERIMENTAL VALUES	THEORETICAL VALUES			
		From Daily Yields	From Weekly Yields		
			Zero-Order Production	1st-Order Production	2nd-Order Production
13th-night dent	17.90	22.1	23.3	21.2	20.1
13th-night sweet	18.40	24.5	26.5	20.1	18.1
20th-night dent	23.15	27.4	24.8	26.1	30.0
20th-night sweet	22.50	33.4	26.1	28.0	37.4

The above calculations can also be made, assuming that amylopectin is produced during the daytime and amylose during the night. When this is done, the theoretical value for the percent amylose for the 13th or 20th night sample is lowered by approximately the same amount as it is raised under the previous assumption. That is, the 13th-night sample of sweet corn starch would have a theoretical value from daily yields of approximately 12.3% amylose as compared to the experimental value of 18.4%, assuming that only amylopectin is produced during the daytime.

The results of these calculations indicate that amylose and amylopectin are produced at the same time. Thus amylose cannot be produced during the daytime or night by inactivating the branching

enzyme. In other words, any assumption that the branching enzyme is inactivated during the daytime or night because of changes in environment would appear to be invalid. Therefore one must either rely on Whelan and Walker's compartment theory (20) or on theories such as the author's (5) which introduce different enzymatic mechanisms.

The data of Table I show clearly that most of the starch (both amylose and amylopectin) is synthesized during the night. This agrees with the results of Puhr and Hume (19), who found that the maximum production of starch in leaves occurs between 7 p.m. and 1 a.m.

The average chain lengths (6) of the 13th-day waxy, 13th-day dent, and 13th-day sweet corn amylopectins are, respectively, 17.2, 14.7, and 12.5 glucose units. Although these are average chain lengths, it is evident that those amylopectins or glycogens having a large average chain length will possess longer and less sterically hindered linear chains. Consequently, the waxy amylopectin should be able to crystallize more readily to form starch granules. This may account for the apparently large amount of starch produced in the waxy corn endosperms during the daytime (see Table I). The amylopectin (or glycogen) in sweet and dent corn endosperms would begin to crystallize in larger amounts during the evening after partial debranching by the proposed debranching enzyme has occurred.

Production of Starch in Potato Tubers. The potato tubers obtained from plants grown in the shade were fewer in number but comparable in size to the normal potatoes. The potato tubers obtained from plants grown under constant light (except for 2 hours) were quite small in size, indicating that large diurnal variations in light (rest periods) are necessary for the production of the tubers.

The samples from plants which were exposed to light at night had 20.25% amylose; those in the shade had 21.65% amylose; and the control samples had 20.95% amylose (see Fig. 2). Thus the variation in both cases was 0.70% amylose from the control. The amounts of potato starch (0.179 g.) used in the iodine titrations were within 0.4% of each other. The moisture in all of these samples was determined under the same conditions and at the same time as described under "Experimental Work." Therefore any large error in the observed percent amylose will be an absolute error and not a relative error. The observed differences in the percentage of amylose in the potato starch samples may therefore be real. At present they cannot be fully explained, but they may be due to a slight change in the activity of the proposed debranching enzyme caused by a change in the growing conditions. If this is true, then the proposed debranching enzyme may be most active (less inhibitor present?) when the plant is exposed to

less light.

The small differences in percentages of potato amylose would appear, however, to rule out the possibility that amylose is synthesized during the daytime and amylopectin during the night (or vice-versa) in potato tubers. The results on the production of amylose in potato tubers are therefore in agreement with the above conclusions concerning the production of corn starch.

Production of Starch in the Bagged and Husked Corn Ears. The effect of water evaporation on the production of starch in the corn endosperm was studied by bagging and husking the ears after pollination (see "Experimental Work"). The results are listed in Table III. The husked samples showed no marked variation in amylose content from the control. They were, however, exposed to the sunlight and their pericarps became extremely hard. This may have prevented a rapid loss of moisture. The percentages of amylose in the bagged samples for dent and sweet corn are much lower than those in the control samples. Wolf *et al.* (21) have shown by both alcohol fractionation and iodine titrations that the percentage of amylose increases with maturity. The iodine titration results listed in Table I and those of Maywald *et al.* (17) are in agreement with the results of Wolf *et al.* (21). Therefore the percentage of amylose in the starch appears to be an

TABLE III

COMPARISON OF STARCH YIELD AND PERCENT AMYLOSE OF CONTROL WITH THAT OF BAGGED AND HUSKED SAMPLES

SAMPLE[a]	TOTAL STARCH YIELD[b]	PERCENTAGE OF AMYLOSE[c]
	CONTROL	CONTROL
Dent corn starch		
13th, Husked	1.50/ 6.67	17.7 /17.9
13th, Bagged	0.13/ 6.67	12.5 /17.9
20th, Husked	19.0 /21.0	24.1 /22.4
20th, Bagged	3.9 /21.0	19.6 /22.4
Sweet corn starch		
13th, Husked	0.56/ 1.16	16.7 /16.1
13th, Bagged	0.18/ 1.16	12.7 /16.1
20th, Husked	5.2 / 5.76	21.9 /23.1
20th, Bagged	0.11/ 5.76	15.2 /23.1
Waxy corn starch		
13th, Husked	2.0 / 5.27	1.1 / 0.92
13th, Bagged	0.58/ 5.27	3.1 / 0.92
20th, Husked	17.0 /26.7	0.87/ 0.68
20th, Bagged	3.4/26.7	0.84/ 0.68

[a] Numbers designate the number of days after pollination. See "Experimental Work" for definition of the terms "husked" and "bagged."

[b] Total starch yield in g. per ear of defatted and dried corn starch samples divided by that of the respective control starch samples.

[c] Percentage of amylose in sample and control obtained by assuming that pure amylose binds 19 mg. of iodine per 100 mg. of sample.

indication of the maturity of the particular corn endosperm.[4] Consequently, the low amylose content of the bagged samples indicates that the growth of their endosperms has been retarded. Also, the immature appearance of the bagged kernels (small and white) and their very small starch yield (see Table III), gives further evidence that bagging the ears retards the growth of the corn endosperm. This may have been caused by either 1) a decrease in the amount of water evaporating from the kernel, or 2) a change in atmospheric conditions surrounding the corn ear. If the former is true, then one might conclude that water evaporation is necessary for rapid crystallization of starch in the corn endosperm and hence for the maturation of the endosperm. From this experiment one can conclude that the absence of amylose in Badenhuizen's (1) incubated young leaves of *Scilla ovatifolia* Bak. was due to a retarding of some of the growth factors in the starch-synthesizing cells.

From the results on samples of sweet and dent corn starch one would expect that bagging the ears of waxy corn plants would also retard the growth of the endosperm. Assuming that this is true, then the results in Table III for the bagged sample of the 13th-day waxy corn starch indicates that amylose is produced by retarding the growth of waxy corn endosperm. This sample stains blue with iodine whereas the control stains brown, indicating that the iodine affinity is not due to some unknown anomaly. There is a possibility that the amylose is due to impurities from the ovary or pericarp. However, such impurities should have manifested themselves in the husked and control waxy corn starch samples. The production of amylose in the more immature cells of waxy corn endosperm was also noted by Lampe (14). The production of amylose in incubated waxy maize endosperm cells as found by Badenhuizen (1) and Fuwa (9) may also be due to retarding the growth of certain factors in the starch-synthesizing cells. These factors may be correlated with the synthesis of an inhibitor to the proposed (5) debranching enzyme. It is postulated that the production of amylose in very immature waxy starch-synthesizing cells is due to less activity or to a destruction of this inhibitor.

Chain Length of Amylose as a Function of the Chain Length of the Corresponding Amylopectin. The ferricyanide numbers of the 20th-day and mature sweet corn starch samples and their approximate number-average degrees of polymerization ($\overline{X}_n$) estimated from other studies (13) are compared with the corresponding chain length (6) of the amylopectin in Table IV. The ferricyanide number from Kerr (13) for

[4] This experimental observation indicates that a starch having 100% amylose content can never be obtained, no matter what the genetic background of the endosperm may be.

mature dent corn amylose and the corresponding $\overline{X}_n$ is also listed in Table IV. The smaller degrees of polymerization of the sweet corn amyloses as compared to the dent corn amylose may be due in part to hydrolysis or oxidation during the isolation. The degradation may however, be slight, since buffered solutions were used (7). Nevertheless,

TABLE IV

NUMBER-AVERAGE DEGREES OF POLYMERIZATION OF THE AMYLOSES COMPARED TO THE AVERAGE CHAIN LENGTH OF THE CORRESPONDING AMYLOPECTIN

CORN STARCH SAMPLE	AMYLOSE FERRICYANIDE NUMBER	$\overline{X}_n$ [a]	CHAIN LENGTH [b] OF AMYLOPECTIN
20th Day sweet	4.17	140	12.2
Mature sweet	2.79	270	16.4
Mature dent	1.43 [c]	455 [c]	25.0

[a] Approximate.
[b] See ref. 6.
[c] See ref. 13.

since both sweet corn amyloses were isolated under the same conditions, the degree of degradation should be approximately the same in both cases. The results, therefore, indicate that the molecular weight of sweet corn amylose increases with an increase in the chain length of the amylopectin.

According to the author's proposed mechanism (5), the synthesis of amylose can be thought of as connecting end-to-end the branches which are removed from the parent glycogen. The average number of branches connected consecutively by the debranching enzyme can be called the "degree of polymerization" of these removed branches. This average "degree of polymerization" would most likely depend on the enzymatic properties of the particular debranching enzyme and the effective number (activity) of receptor groups available to the debranching enzyme-chain complex. If we assume for the sweet and dent corn endosperms that the proposed debranching enzymes have the same properties in all the corn endosperms and that the average "degree of polymerization" is constant, then the molecular weight of the amylose will be a function only of the average length of the removed chain. This would account for the above observed increase in molecular weight of the amyloses with an increase in the average chain length of the corn amylopectins. However, other explanations for this phenomenon could also be used without employing the author's (5) proposed mechanism. For example, assuming that the amylose is the precursor of amylopectin, then the optimum chain length for phosphorylase (or whatever enzyme system is involved in the synthesis of straight chains) could increase with maturity. Nevertheless, it is pointed

out that the results do not contradict the author's (5) proposed mechanism for the synthesis of starch from glycogen.

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Literature Cited

1. Badenhuizen, N. P. Distribution of acid phosphatase and phosphorylase in relation to starch production. Acta Botanica Neerl. **4:** 565–574 (1955).
2. Barker, S. A., and Bourne, E. J. Enzymatic synthesis of polysaccharides. Quart. Revs. (London) **7:** 56–83 (1953).
3. Bates, F. L., French, D., and Rundle, R. E. Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. **65:** 142–148 (1943).
4. De Duve, C., and Hers, H. G. Carbohydrate metabolism. Ann. Rev. Biochem. **26:** 149–180 (1957).
5. Erlander, S. R. A proposed mechanism for the synthesis of starch from glycogen. Enzymologia **29:** 273–283 (1958).
6. Erlander, S. R., and French, D. Acid hydrolysis and molecular weight of various corn amylopectins and glycogen. J. Polymer Sci. **32:** 291–316 (1958).
7. Erlander, S. R,. and French, D. Dispersion of starch granules and the validity of light scattering results on amylopectin. J. Am. Chem. Soc. **80:** 4413–4420 (1958).
8. Ewart, M. H., Siminovitch, D., and Briggs, D. R. Studies on the chemistry of the living bark of the black locust in relation to its frost hardiness. VII. Possible enzymatic processes involved in starch-sucrose interconversions. Plant Physiol. **29:** 407–413 (1954).
9. Fuwa, H. Formation of starch in young maize kernels. Nature **179:** 159–160 (1957).
10. Greenwood, C. T. Aspects of the physical chemistry of starch. *In* Advances in carbohydrate chemistry, vol. 11, p. 335. Academic Press: New York (1956).
11. Hehre, E. J. Synthesis of a polysaccharide of the starch-glycogen class from sucrose by a cell-free, bacterial enzyme system (amylosucrase). J. Biol. Chem. **177:** 267–279 (1949).
12. Hobson, P. N., Whelan, W. J., and Peat, S. The enzymic synthesis and degradation of starch. Part XII. The mechanism of synthesis of amylopectin. J. Chem. Soc. **1951:** 596–598.
13. Kerr, R. W. Chemistry and industry of starch (2nd ed.), pp. 188 and 679. Academic Press: New York (1950).
14. Lampe, Lois. A microchemical and morphological study of the developing endosperms of maize. Bot. Gaz. **91:** 337–376 (1931).
15. Lansky, Sylvia, Kooi, Mary, and Schoch, T. J. Properties of the fractions and linear subfractions from various starches. J. Am. Chem. Soc. **71:** 4066–4075 (1949).
16. Manners, Dorothea J. The enzymatic degradation of polysaccharides. Quart. Revs. (London) **9:** 73–99 (1955).
17. Maywald, Eileen, Christensen, Ruth, and Schoch, T. J. Development of starch and phytoglycogen in golden sweet corn. J. Agr. Food Chem. **3:** 521–523 (1955).
18. Nussenbaum, S., and Hassid, W. Z. Mechanism of amylopectin formation by the action of Q enzyme. J. Biol. Chem. **196:** 785–792 (1952).
19. Puhr, L. F., and Hume, A. N. Variations in amounts of carbohydrates in the leaves of corn. So. Dak. Agr. Exp. Sta. Bull. 270 (1932).

20. WHELAN, N. J., and WALKER, G. J. A multi-enzyme system for the concurrent synthesis of amylose and amylopectin. Abstr. of Communications. IV. International Congress of Biochem., Vienna, Sept. 1–6, 1958, p. 46.

21. WOLF, M. J., MACMASTERS, MAJEL M., HUBBARD, J. E., and RIST, C. E. Comparison of corn starches at various stages of kernel maturity. Cereal Chem. 25: 312–325 (1948).

DETERMINATION OF URIC ACID IN WHEAT FLOUR INFESTED BY TRIBOLIUM CASTANEUM DUV., USING PAPER CHROMATOGRAPHY[1]

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ABSTRACT

The uric acid present in infested wheat flour was separated by paper chromatography and quantitatively estimated using Benedict's uric acid reagent. The results agreed with those obtained by a direct colorimetric method of determining uric acid in protein-free aqueous extracts of infested flour. The uric acid content in the infested flour increased steadily with the progress of infestation. No uric acid could be detected in the extract of control uninfested flour. The protein-free aqueous extract of control uninfested wheat flour, however, contained small amounts of substances (other than uric acid) which reacted with the Benedict's uric acid reagent and yielded an "apparent" uric acid value. This value was low, as compared with those found in the infested flour.

Earlier investigations from this laboratory (5,7,8) have shown that uric acid, which is an important constituent in the excreta of insects, can serve as a good index of the degree of infestation and unhygienic conditions in infested food grains. In the above studies, uric acid was determined in the protein-free aqueous extracts of foodstuffs by a colorimetric method using Benedict's uric acid reagent (3). Protein-free aqueous extracts of uninfested food samples also contained relatively small amounts of substances which reacted with the uric acid reagent and gave some "apparent" uric acid values. In view of the fact that Benedict's reagent is not specific for uric acid (3), it was felt desirable to determine the uric acid content of infested foods using a more specific method. In the present investigation, the uric acid content of wheat flour infested by *Tribolium castaneum* Duv. has been determined by paper chromatography and the results have been compared with those obtained by direct colorimetric method.

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Materials and Methods

The samples of wheat flour infested by *Tribolium castaneum* Duv. and the control uninfested wheat flour used in the present study were the same as those described in the preceding paper (9).

Determination of Uric Acid in Wheat Flour by Direct Colorimetry. The method used in the present study is a slight modification of that described earlier (5) and is briefly described below: Wheat flour (5–20 g.), containing about 1–5 mg. of uric acid, was suspended in 200 ml. of water. The mixture was allowed to stand for 2 hours with occasional stirring, and then mixed in a Waring Blendor for 10 minutes. It was centrifuged at 2000 r.p.m. for 10 minutes. To 100 ml. of the clear centrifugate, 10 ml. of 10% sodium tungstate were added. After mixing, 10 ml. of 0.667*N* sulfuric acid were added to precipitate the proteins present in the extract. The mixture was allowed to stand for 5 minutes and then filtered. Aliquots of the filtrate containing about 50–100 γ of uric acid were used for the colorimetric determination according to Hawk *et al.* (3). The color intensity was determined in a Klett-Summerson photoelectric colorimeter with 520 $m\mu$ filter.

Determination of Uric Acid by Paper Chromatography. Tilden (6) used the paper chromatographic technique for separation and estimation of uric acid in infested fruit products. Johnson (4) and Dikstein *et al.* (2) have described paper-chromatographic techniques for the assay of uric acid in urine. After a preliminary study of the different solvent systems suggested by the above authors, a simple solvent system consisting of 1-butanol-acetic acid-water (4:1:5) was found to be quite satisfactory for the separation of uric acid from extracts of infested wheat flour. The paper-chromatographic procedure finally adopted was as follows:

Extraction of Uric Acid. Wheat flour (10–20 g.) containing about 1–10 mg. of uric acid (as determined by direct colorimetric assay) was suspended in 100 ml. of water at room temperature (24°–29°C.). The mixture was allowed to stand for 2 hours with frequent stirring and was then mixed thoroughly for 10 minutes in a Waring Blendor. It was then centrifuged at 2000 r.p.m. for 15 minutes. The centrifugate was filtered and 20 ml. of the clear filtrate were evaporated on a water bath, so that 1 ml. of concentrated extract contained about 0.1–0.2 mg. of uric acid.

Paper-Chromatographic Technique. The separation of uric acid from the extracts of wheat flour was effected by the descending paper chromatographic technique on Whatman No. 1 paper using 1-butanol-acetic acid-water (4:1:5). The chromatographic chamber was similar

to that described by Block and Bolling (1). Known volumes of the concentrated extract of wheat flour containing about 10–15 γ of uric acid were spotted at intervals of about 4 cm. on Whatman No. 1 filter paper (45–50 cm. long and 18 cm. wide) on a line drawn about 10 cm. from one end of the paper. A total of 50–100 μl. of the extract could be applied to one spot, by repeatedly spotting in 10-μl. quantities and drying the spot in a current of warm air. Known quantities of standard uric acid (15 γ) were also spotted next to each unknown spot. The filter paper sheet was suspended from a glass trough containing the solvent mixture and fitted near the top of a rectangular glass chamber (1). The whole assembly was kept at room temperature (24°–29°C.), and the chromatogram was allowed to develop for 48 hours. At the end of this period, the chromatograms were removed and dried at 50°C. and sprayed with a saturated solution of sodium carbonate followed by arsenophosphotungstic acid reagent, according to Johnson (4). Blue spots were obtained at points where uric acid was located. The R_f value of uric acid was found to be 0.41 in the solvent mixture used. Using the developed chromatogram as a guide, the positions of uric acid spots were located in the untreated portion of the chromatogram. The areas corresponding to that of the uric acid spots were marked with pencil allowing ample space all around the spot, and the spots were cut out. Each cut strip was placed in a test tube and the uric acid present was extracted twice with 5 ml. of phosphate buffer (pH 6.8) at 60°C., and filtered. The uric acid in the filtrate was determined colorimetrically, as in the direct method, according to Hawk *et al.* (3) after 4 ml. of sodium cyanide solution and 1 ml. of Benedict's uric acid reagent were added.

Results and Discussion

Table I shows that the results obtained by the two methods are in close agreement. The results of chromatographic studies also showed that only one spot corresponding to the standard uric acid, with R_f value of 0.41, could be observed in the chromatogram obtained from the extract of infested wheat flour. No uric acid could be detected in the chromatogram developed with the extract of control wheat flour. The recovery of uric acid added to control and infested flours was of the order of 90–100%. In the present study an "apparent" uric acid value of about 5 mg. per 100 g. was found in the extract of control wheat flour by the direct colorimetric method. Since no uric acid could be found in the extract by paper chromatography, this "apparent" uric acid value is due to the presence of other substances which react with Benedict's uric acid reagent. In view of the fact that the

TABLE I

URIC ACID CONTENT OF WHEAT FLOUR INFESTED BY *Tribolium castaneum Duv.*, DETERMINED BY TWO METHODS[a]

PERIOD OF STORAGE	UNINFESTED WHEAT FLOUR		INFESTED WHEAT FLOUR		
	Direct Colorimetric Procedure	Paper Chromatography	Direct Colorimetric Procedure		Paper Chromatography
			Total Uric Acid	Corrected Uric Acid[b]	
months					
0	5.8	nil	5.7	nil	nil
1	5.4	nil	16.0	10.3	12.8
2	4.6	nil	43.9	38.2	37.5
3	5.2	nil	76.0	70.3	73.2
4	4.8	nil	112.7	107.0	105.4
5	4.5	nil	161.4	155.7	159.4

[a] All uric acid values have been expressed as mg. per 100 g. of flour on 14% moisture basis.
[b] Corrected uric acid values were obtained by subtracting "apparent" uric acid value of the uninfested flour at the beginning of the experiment from the total uric acid values.

"apparent" uric acid values obtained for unifested grains are low (5,7,8) as compared with the high uric acid values generally found in infested grains, the direct colorimetric determination of uric acid described in this paper can be used for the routine assay of the uric acid content of infested cereals and cereal products.

Literature Cited

1. BLOCK, R. J., and BOLLING, DIANA. The amino acid composition of proteins and foods (2nd ed.), p. 410. Charles C. Thomas: Springfield, Illinois (1951).
2. DIKSTEIN, S., BERGMANN, F., and CHAIMOVITZ, M. Studies on uric acid and related compounds. II. Paper chromatography of substituted xanthines and uric acid. J. Biol. Chem. **221:** 239–251 (1956).
3. HAWK, P. B., OSER, B. L., and SUMMERSON, W. H. Practical physiological chemistry (12th ed.), p. 844. The Blakiston Co.: Philadelphia (1947).
4. JOHNSON, E. A. The occurrence of substituted uric acids in human urine. Biochem. J. **51:** 133–138 (1952).
5. SUBRAHMANYAN, V., SWAMINATHAN, M., PINGALE, S. V., and KADKOL, S. B. Uric acid as an index of insect filth in cereals and milled cereal products. Bull. Central Food Technol. Research Inst., Mysore (India) **4:** 86–87 (1955).
6. TILDEN, DORIS H. Report on uric acid in fruit products. J. Assoc. Offic. Agr. Chemists **34:** 498–505 (1951).
7. VENKATRAO, S., NUGGEHALLI, R. N., PINGALE, S. V., SWAMINATHAN, M., and SUBRAHMANYAN, V. The relation between the uric acid content and the extent of kernel damage in insect-infested grain. Food Sci. **6:** 273–275 (1957).
8. VENKATRAO, S., NUGGEHALLI, R. N., SWAMINATHAN, M., PINGALE, S. V., and SUBRAHMANYAN, V. Effect of insect infestation on stored grain. III. Studies on kaffir corn (*Sorghum vulgare*). J. Sci. Food Agr. **9:** 837–839 (1958).
9. VENKATRAO, S., NUGGEHALLI, R. N., PINGALE, S. V., SWAMINATHAN, M., and SUBRAHMANYAN, V. The effect of infestation by *Tribolium castaneum* Duv. on the quality of wheat flour. Cereal Chem. **37:** 97–103 (1960).

THE EFFECT OF INFESTATION BY TRIBOLIUM CASTANEUM DUV. ON THE QUALITY OF WHEAT FLOUR[1]

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ABSTRACT

The changes occurring in hard Indian wheat flour (75% extraction) subjected to infestation by *Tribolium castaneum* Duv., in 4-gal. tins provided with lever lids, at a temperature of 85° ± 5°F. and a relative humidity of 70–75%, were studied during a period of 5 months. The uric acid content (derived from insect excreta) of the infested flour was proportional to the insect population and served as a good index of the unhygienic condition in the flour due to the presence of insect excreta. A marked increase in fat acidity and a decrease in the thiamine content were observed in the infested flour. The gluten obtained from flour infested for 4 to 5 months was brittle. The values for the wheat meal time test gradually decreased with the progress of infestation, indicating a deterioration in the quality of gluten. The loaf volume of the bread also gradually decreased as the infestation progressed. Organoleptic evaluation revealed that bread made from flour infested for more than 1 month had an off-flavor and bitter taste and was not acceptable. The uninfested control flour remained in good condition throughout, and the bread made from it was quite acceptable.

Several flour storage studies have been reported in the literature, but in most of the earlier studies no attempt has been made to follow the biochemical changes during storage. References to important studies on the subject have been given by Cuendet *et al.* (4) and Greer *et al.* (5). Cuendet *et al.* (4) reported a detailed study of the influence of moisture, temperature, and other factors on the keeping quality of flour. They found that when flours having moisture contents of 10 and 14% were stored at 37.8°C., a marked reduction in loaf volume occurred at the end of 38 and 10 weeks respectively. Under conditions prevailing in India and other tropical countries, wheat flour is frequently infested by insects. No information is available in the literature on the changes brought about by insect infestation on the quality of flour. The present paper deals with studies on the changes in the chemical composition and the baking quality of wheat flour infested by *Tribolium castaneum* Duv. during storage.

Materials and Methods

Materials. Wheat flour (75% extraction) prepared from Indian hard wheat was obtained from a mill in Bangalore within a week after milling. The material contained 10.6% moisture and was stored in tins

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of 4-gal. capacity provided with lever lids. Each tin contained 15 lb. flour and the lid was not air-tight. One lot of six tins was infested by introducing 100 adults of *Tribolium castaneum* Duv. in each tin. A second lot of six tins was kept free from infestation and served as control. All the tins were stored for 5 months in a room maintained at 85° ± 5°F. and relative humidity of 70–75%. At monthly intervals 1 lb. of the flour was removed from each tin after thorough mixing. The samples from three tins were mixed and the two lots of mixed samples were used for analysis. The infested flour samples were passed through a 50-mesh sieve to remove the insects and the sieved flour was used for the analysis and baking trials. Baking trials and determination of insect population and uric acid content in the samples were carried out at monthly intervals. The chemical analysis of the samples was conducted once in every 2 months.

Methods. For the determination of insect populations only larval, pupal, and adult stages of the insect were counted.

Nitrogen, moisture, fat acidity, crude gluten, and wheat meal time test were determined according to the methods of the American Association of Cereal Chemists (1).

Fat was determined according to the method of AOAC (2).

Uric acid content of the infested flours was determined by the method of Subrahmanyan *et al.* (7) as modified by Venkatrao *et al.* (10).

Water-soluble nitrogen and acetic acid-soluble nitrogen were estimated according to the method of Sollars (6).

Nitrogen soluble in 60% ethanol (gliadin N) was determined as follows: flour (10 g.) was suspended in 250 ml. of water and the mixture was stirred for 15 minutes and centrifuged for 10 minutes at 2000 r.p.m. The supernatant was discarded, and the residue mixed with 200 ml. of 65% alcohol, stirred for 6 hours, allowed to remain overnight, and centrifuged. Aliquots of the alcoholic extracts were used for nitrogen assay.

Nonprotein nitrogen was determined as follows: 10 g. of the flour were treated with 100 ml. of 5% trichloroacetic acid for precipitating the proteins. The mixture was shaken for 15 minutes and filtered. The nitrogen content in aliquots of the filtrate was determined.

Thiamine was determined by the thiochrome method (9).

The baking test was conducted in a local bakery using 450 g. of the flour samples. The formula contained 1% salt, 1.5% yeast, and an appropriate quantity of water. The doughs were fermented 2 hours at 30°C., proofed 1 hour at 30°C., and baked for 25 minutes at 230°C. The acceptability of the bread prepared from stored and infested flour was determined with the help of a panel of six judges selected from the

staff members of the Institute. For the assessment of off-flavor present, the following scoring system was used: 0, free from off-flavor and highly acceptable; 1, a suspicion of off-flavor but palatable; 2, slight off-flavor and bitterness, and unpalatable; 3, marked off-flavor and bitterness, and unpalatable; 4, very marked off-flavor and bitterness, and highly unpalatable.

Results and Discussion

Data regarding the insect population, insect fragment count, and uric acid content in infested flours are presented in Table I. The results of chemical analysis are given in Table II. The results of baking tests are shown in Table III and in Fig. I.

TABLE I

INSECT POPULATION, INSECT FRAGMENT COUNT, AND URIC ACID CONTENT OF STORED WHEAT FLOUR (75% EXTRACTION) INFESTED BY TRIBOLIUM CASTANEUM DUV.

PERIOD OF INFESTATION	INSECT POPULATION[a]		INSECT FRAGMENT COUNT[a]	URIC ACID[b]
	Adults	Larvae and Pupae		
months				*mg/100 g*
1	27	162	1692	10.3
2	128	371	2716	38.2
3	148	1192	3825	70.3
4	198	1076	3042	107.0
5	250	958	2817	155.7

[a] Values expressed per lb. of flour on 14% moisture basis.
[b] Uric acid values expressed on 14% moisture basis for the flour.

Insect Count and Uric Acid Content. Table I shows that the adult and larval counts of insects in flour increased up to a period of 4 months. The larval population showed a decrease by the fifth month. The insect fragment count also increased up to 3 months, after which a slight decrease in the count was observed. The insect fragments are mainly the cuticle derived from the immature stages of the insects during their development.

Though the insects and larvae were removed as a result of screening through a 50-mesh sieve, the excreta and fragments are still associated with infested flour. Recent investigations have shown that uric acid, which is the main end-product of nitrogen metabolism of insects, can serve as a good index of the unhygienic condition in infested cereals and pulses (legumes) (7,11,12). The data in Table I show that uric acid content can serve as a good index of insect filth in infested flour as well. No uric acid could be detected in the uninfested control samples by paper-chromatographic technique (10). The results show that even if

TABLE II

CHANGES IN CHEMICAL COMPOSITION DURING STORAGE OF WHEAT FLOUR (75% EXTRACTION) INFESTED BY TRIBOLIUM CASTANEUM DUV., AS COMPARED WITH UNINFESTED FLOUR[a]

CONSTITUENT	CONTROL (INSECT-FREE) — MONTHS OF STORAGE				INFESTED FLOUR — MONTHS OF STORAGE		
	0	2	4	5	2	4	5
Moisture, %	10.6	10.6	10.8	10.9	10.8	11.5	13.1
Total nitrogen, %	1.524	1.522	1.526	1.520	1.526	1.610	1.625
Nonprotein nitrogen[b]	5.5	5.9	6.2	6.6	7.2	11.3	14.8
Nitrogen, soluble in water[b]	14.6	15.4	15.9	15.2	18.8	23.0	25.4
Nitrogen, soluble in dilute acetic acid[b]	77.3	76.6	75.0	74.3	67.2	63.6	58.3
Nitrogen, soluble in 60% alcohol[b]	42.2	42.7	41.0	40.8	40.7	38.0	36.2
Crude gluten, %	7.4	7.3	7.2	7.3	6.8	5.0[d]	4.2[d]
Wheat meal time test (minutes)	148	145	144	140	132	112	93
Fat, %	1.15	1.08	1.09	1.10	1.02	0.83	0.74
Thiamine, γ per 100 g.	160	160	152	146	153	92	76
Fat acidity[c]	66	81	102	120	149	199	225

[a] All analytical values are expressed on 14% moisture basis.
[b] Expressed as % of total nitrogen.
[c] Expressed as mg. KOH per 100 g. flour.
[d] The gluten was brittle and was partly lost during washing.

infested flour is fumigated and cleaned of insects and sold in the market, the uric acid content can very well serve as a good index of the degree of unhygienic conditions in the material. Flour infested for periods longer than 4 months turned slightly yellow and developed off-flavor as a result of accumulation of insect excreta.

Changes in Chemical Composition. The moisture content of the infested flour increased as the infestation progressed. A marked in-

TABLE III

LOAF VOLUME AND CONSUMER ACCEPTABILITY OF BREAD PREPARED FROM CONTROL AND INFESTED WHEAT FLOUR (75% EXTRACTION)

PERIOD OF STORAGE	LOAF VOLUME		ORGANOLEPTIC ACCEPTABILITY SCORE [a]	
	Control	Infested	Control	Infested
months	*cc*	*cc*		
0	2680	2680	0	0
1	2650	2500	0	1
2	2640	2420	0	2
3	2630	2340	0	3
4	2660	2250	0	4
5	2620	2130	0	4

[a] Details of organoleptic score are given under "Methods."

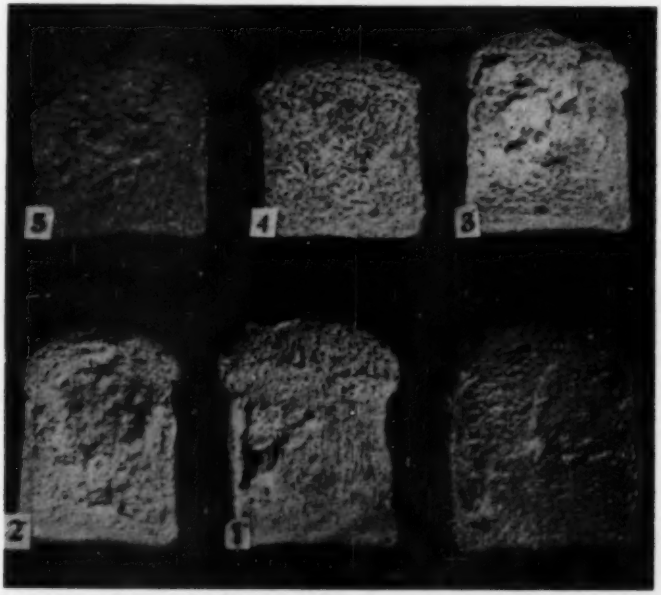


Fig. 1. Cut surfaces of bread from wheat flour: uninfested control (no number) stored 5 months; and infested flour stored 5, 4, 3, 2, and 1 month.

crease in the nonprotein nitrogen was observed in the infested samples. This may be partly due to the nitrogenous constituents present in insect excreta and partly the result of proteolysis. An appreciable reduction was observed in the nitrogen soluble in dilute acetic acid in the infested flour, indicating a decrease in the gluten content due to its partial breakdown into nonprotein nitrogen. An appreciable loss of thiamine occurred in the infested flour. Fat acidity increased markedly with the progress of infestation. Gluten present in flour infested for 4 to 5 months was brittle and disintegrated easily. A part of it was lost during washing, thus lowering the gluten yield. Sullivan *et al.* (8) and Barton-Wright (3) reported that unsaturated fatty acids produced by the hydrolysis of fat present in wheat flour rendered the gluten short and brittle. Infestation caused a decrease in the time taken for dough balls to disintegrate in water, thereby indicating deterioration in gluten quality.

Baking Test. Table III shows that insect infestation affected the baking quality of the flour. The loaf volume of infested flour was reduced and the crumb was compact and inelastic. Organoleptic tests showed that bread prepared from the flour infested for more than 1 month had a bitter taste and off-flavor and was not acceptable to the average consumer. The loaf volume of the bread made from the uninfested control flour was, however, not affected and the bread was quite acceptable to the consumers.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). The Association: St. Paul, Minnesota (1947).
2. Association of Official Agricultural Chemists. Official methods of analysis (7th ed.). Assoc. Offic. Agr. Chemists: Washington D.C. (1950).
3. Barton-Wright, E. C. Studies on the storage of wheaten flour. III. Changes in the flora and the fats and the influence of these changes on gluten character. Cereal Chem. **15**: 521–540 (1938).
4. Cuendet, L. S., Larson, E., Norris, C. G., and Geddes, W. F. The influence of moisture content and other factors on the stability of wheat flours at 37.8°C. Cereal Chem. **31**: 362–389 (1954).
5. Greer, E. N., Jones, C. R., and Moran, T. The quality of flour stored for periods up to 27 years. Cereal Chem. **31**: 439–450 (1954).
6. Sollars, F. W. A new method of fractionating wheat flour. Cereal Chem. **33**: 111–120 (1956).
7. Subrahmanyan, V., Swaminathan, M., Pingale, S. V., and Kadkol, S. B. Uric acid as an index of insect filth in cereals and milled cereal products. Bull. Central Food Technol. Research Inst., Mysore (India) **4**: 86–87 (1955).
8. Sullivan, Betty, Near, Cleo, and Foley, G. H. The role of lipids in relation to flour quality. Cereal Chem. **13**: 318–331 (1936).
9. Swaminathan, M. An improved method for the estimation of vitamin B_1 in foods by the thiochrome reaction. Indian J. Med. Research **30**: 263–272 (1942).
10. Venkatrao, S., Krishnamurthy, K., Swaminathan, M., and Subrahmanyan, V. Determination of uric acid in wheat flour infested by *Tribolium castaneum* Duv., using paper chromatography. Cereal Chem. **37**: 93–96 (1960).

11. VENKATRAO, S., NUGGEHALLI, R. N., PINGALE, S. V., SWAMINATHAN, M., and SUBRAHMANYAN, V. The relation between the uric acid content and the extent of kernel damage in insect infested grain. Food Sci. 6: 273–275 (1957).
12. VENKATRAO, S., NUGGEHALLI, R. N., SWAMINATHAN, M., PINGALE, S. V., and SUBRAHMANYAN, V. Effect of insect infestation on stored grain. III. Studies on Kaffir corn (*Sorghum vulgare*). J. Sci. Food Agr. 9: 837–839 (1958).

A NOTE ON THE VITAMIN B CONTENT OF KOREAN RICE[1]

C. H. BAILEY

In a country such as Korea, where much of the rice destined for human food is milled in small roadside or village rice mills, the issue of the degree of refinement and consequent vitamin content of the milled rice is of significance. Accordingly, it appeared desirable to collect and analyze a sufficient number of typical samples of Korean milled rice to determine that variability, as well as the average of the content of certain constituents. The resulting data might thus serve as a basis for appraising the adequacy of the Korean diet and the possible desirability of some supplementation, as, for example, the enrichment of such rice.

A summary of the analytical data resulting from this study is recorded in Table I. It appears that the thiamine content of these Korean rice samples is about double that reported by Geddes (1) from the data of Kik and Van Landingham (2,3,4), and of Williams, Knox, and Fieger (6). The riboflavin content of the Korean samples is slightly, although significantly, higher than that reported by Kik *et al.* (2,3,4), while the niacin content is in approximately the same range.

The average thiamine content of the milled rice of Far Eastern countries reported by Woot-Tsuen Wu Leung *et al.* (5) was 1.2 parts per million, which is about double that reported by Geddes (1), and in the same range as the 1.39 p.p.m found in the regular rice collected from Korean mills. Likewise the ash and crude protein content of the composites of the Korean rice samples were quite similar to the rice studied by Leung *et al.* (5). It appears, therefore, that the relative degree of refinement of the Korean rice is equivalent to that of rice

[1] Manuscript received May 4, 1959. Scientific Journal Series No. 4124, Minnesota Agricultural Experiment Station, St. Paul, Minn.

TABLE I

COMPOSITION OF KOREAN MILLED RICE

	REGULAR RICE FROM MILLS, 18 SAMPLES	"SOFT TYPE" RICE FROM MILLS, 8 SAMPLES	STREET MARKET RICE, 30 SAMPLES
	ppm	*ppm*	*ppm*
Thiamine			
Minimum	1.12	1.54	1.34
Maximum	1.83	1.83	1.83
Average	1.39	1.68	1.54
Riboflavin			
Minimum	0.33	0.35	0.33
Maximum	0.44	0.44	0.53
Average	0.38	0.40	0.40
Niacin			
Average	13.4	15.4	23.1
	%	%	%
Crude protein (N × 5.95)			
Average, 14% moisture basis	6.43	6.54	6.37
Ash			
Average, 14% moisture basis	0.65	0.97	0.58

of the Far Eastern countries generally. Accordingly, substantial rice enrichment might well be applied in Korea, as has been done in other areas of the Orient, in order to include a reasonably adequate supply of certain essential B vitamins, notably thiamine and niacin, in the Korean diet.

Acknowledgments

Grateful acknowledgment is made of the efficient services of Professor Roy O. Bridgford, Over-All Advisor in Agriculture to Seoul National University of Korea under the University of Minnesota's contract with the U. S. International Cooperation Administration, who collected the 56 samples of rice from Korean rice mills and street markets; also of Merck & Co., Inc., of Rahway, N. J., whose experienced analysts determined the vitamin content of the Korean rice samples included in this study.

Literature Cited

1. GEDDES, W. F. Cereal grains. *In* The chemistry and technology of food and food products, ed. by M. B. Jacobs; vol. I, chap. XVII, tables 133 and 134. Interscience: New York (1944).
2. KIK, M. C. Thiamin in products of commercial rice milling. Cereal Chem. **20:** 103–109 (1943).
3. KIK, M. C., and VAN LANDINGHAM, FLOY B. Riboflavin in products of commercial rice milling and thiamin and riboflavin in rice varieties. Cereal Chem. **20:** 563–569 (1943).
4. KIK, M. C., and VAN LANDINGHAM, FLOY B. Nicotinic acid in products of commercial rice milling and in rice varieties. Cereal Chem. **21:** 154–158 (1944).
5. LEUNG, WOOT-TSUEN WU, PECOT, R. K., and WATT, B. K. Composition of foods used in Far Eastern countries. U. S. Dep't Agr., Handbook No. 34 (March 1952).
6. WILLIAMS, VIRGINIA R., KNOX, W. C., and FIEGER, E. A. A study of some of the vitamin B-complex factors in rice and its milled products.. Cereal Chem. **20:** 560–563 (1943).

NOTE ON A METHOD OF APPRAISING MIXOGRAM DATA[1]

SAUL ZALIK AND MIKE OSTAFICHUK

The usual method for recording mixogram results as areas tends to obscure some of the relevant data on the quality of the doughs being studied. The rapid method for estimating mixogram areas described by Yamazaki (3) also has this shortcoming. Consequently, there must be actual comparison of mixograms in evaluating a series of doughs. To resolve this difficulty, we have adopted the following method of scoring mixograms.

A scale with cm. subdivisions, as shown in Fig. 1, is drawn on transparent plastic. To draw the parallel arcs in this scale, a radius equal to that of the pen arm of the mixograph is used. The protractor on this scale is used to measure the angle of the curve at the peak.

The scale is superimposed over a mixogram, and the following readings are taken (Fig. 2): A, angle of curve at peak; 1, number of cm. from starting point to the point of intersection on the base line by the arc which passes through peak of mixogram curve; 2, length in cm. of perpendicular from base to peak; 3, length in cm. of perpendicular from base line at t to curve.

Table I compares our scores with the areas for the group of mixograms shown in Fig. 3.

TABLE I

A COMPARISON OF THE AREAS AND SCORES FOR THE MIXOGRAMS OF FIG. 3

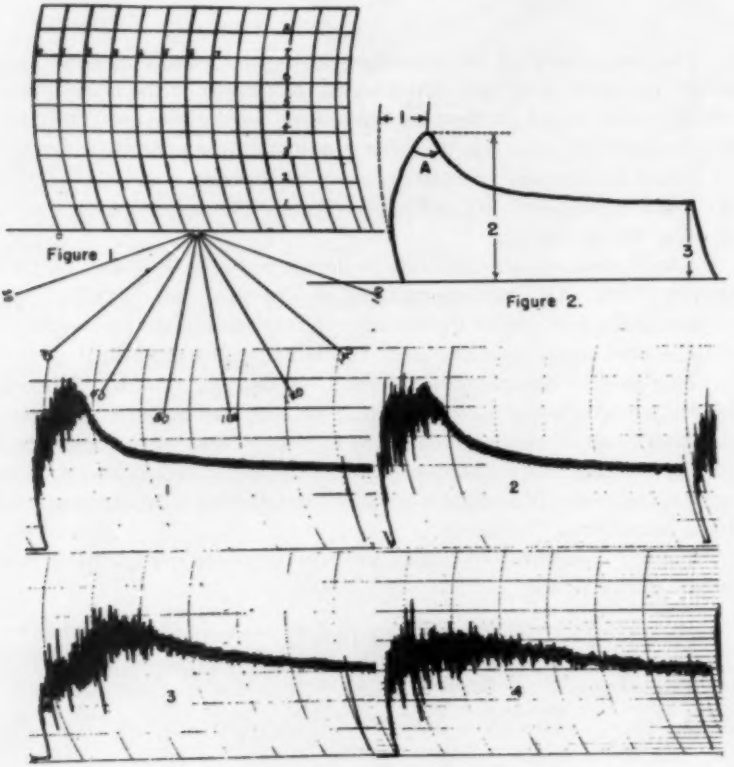
MIXOGRAM NUMBER	MIXOGRAM AREA	SCORES [a]			
		A	1	2	3
	cm^2				
1	45.6	60	2.0	6.0	3.1
2	45.4	90	2.2	5.8	3.0
3	45.5	120	3.8	4.8	3.3
4	45.5	170	4.0	4.3	3.2

[a] A recorded in degrees; 1, 2, and 3 recorded in cm., 3 measured at t (7 minutes).

The method of recording and reporting mixograph data presented here is rapid, and it makes visual comparison of mixograms unnecessary. In addition, it records some of the features of the mixogram which are assumed to represent specific quality characteristics of the dough as defined by Swanson and co-workers (1,2): a) the rate of dough development; b) the maximum extent of the dough's resistance to mechanical

[1] Manuscript received November 17, 1958. Department of Plant Science, University of Alberta, Edmonton, Alberta.

stress; and c) the resistance of the dough to stress after a specified time interval. We found this method expedient in assessing a series of over 300 soft wheat flours.



Figs. 1, 2, and 3. Fig. 1: Scale drawn on transparent plastic. The lines are 1 cm. apart, and protractor divisions 20°. One minute = 1.8 cm. on the base line. Fig. 2: A, 1, 2, and 3 are the measurements recorded. Number 3 is measured at point t, in this case 7 minutes from the starting point. Fig. 3: A series of mixograms. Descriptions of these are given in Table I.

Literature Cited

1. JOHNSON, JOHN A., SWANSON, C. O., and BAYFIELD, E. G. The correlation of mixograms with baking results. Cereal Chem. **20**: 625–644 (1943).
2. SWANSON, C. O., and JOHNSON, JOHN A. Description of mixograms. Cereal Chem. **20**: 39–42 (1943).
3. YAMAZAKI, WILLIAM T. Note on a rapid method for estimation of mixogram area. Cereal Chem. **24**: 518–520 (1947).

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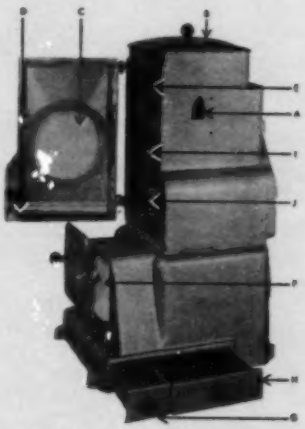

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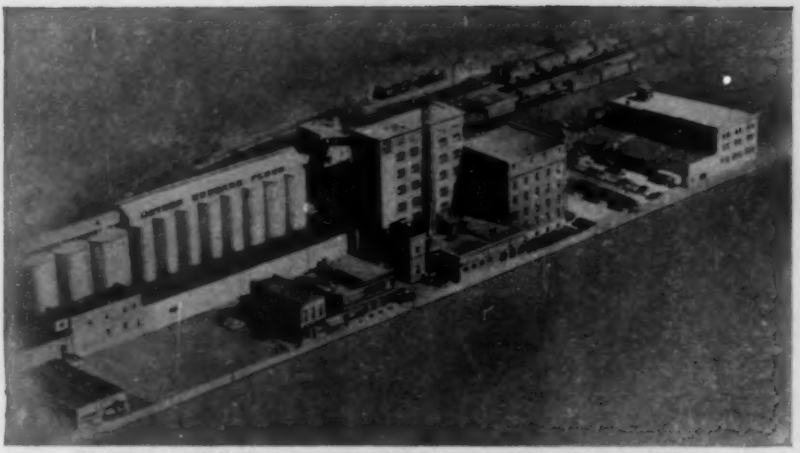

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